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EVALUATION OF PROCESSING VARIABLES TO INCREASE THE
SOLIDS OF MILK PROTEIN CONCENTRATES PRIOR TO DRYING

BY

ACHYUT MISHRA

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Biological Sciences

Specialization in Dairy Science

South Dakota State University

2020

DISSERTATION ACCEPTANCE PAGE

ACHYUT MISHRA

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I am dedicating this dissertation to my beloved mom, Late Januka Devi Mishra.

ACKNOWLEDGEMENTS

I would like to thank my wife and daughter for their patience, love, and continuous support. Lot of thanks to my dad, sisters, brothers, and parents in law for their support and strength. I am grateful to my friends Suvash Kafley and Subharoj Dahal for motivating me for the Ph.D. studies.

I would like to thank Dr. Lloyd Metzger as my major advisor for providing the critical insights and motivation throughout my research works. It is a great privilege for me to work in his team. His choice to let the student creative freedom but with constant good advices always allows to deliver our best. I wish I could be as energetic and enthusiastic as Dr. Metzger during my career. Sincere thanks go to my other advisory committee members 1. Dr. Vikram Mistry for his motivation and providing valuable information, 2. Dr. Sanjeev Anand for the valuable discussion related to microbiological assessments in my studies, and 3. Dr. Christine Stewart for serving as GFR.

Thanks Milk Specialties Global for providing Plate and Frame unit for my research. I also want to thank Dr. J. Amamcharla and Dr. K. Muthukumarappan for helping in valuable discussion. I would like to thank Steve, John, Raunak, Sagar, Vijay, Ahmed, Aki, Dipak, Hiral, Venkat, Neha, Shayanti, Pratishtha, Nancy, Maryam, Pratibha, Khalid, Kaavya, Yasitha, Dilumina, Hyari, Sajeew, Manuja, Hiran and Leyby for helping and creating friendly environment in the department and Davis Dairy Plant. Thanks Jayne and Kristi for helping in official works. I am also thankful to all the administrative staffs of international affairs and graduate school. I am grateful to the Dairy Management Inc. for providing fund for this research. Finally, I want to thank SDSU for providing opportunity to pursue my Ph.D. with great research facilities.

-Achyut

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ABBREVIATIONS

BSA	Bovine serum albumin
°C	Degree Celsius
CE	Capillary electrophoresis
CFU	Colony-forming unit
cP	Centipoise
d	Day
DF	Diafiltration
g	Gram
G'	Storage modulus
h	Hour
HC	Hydrodynamic cavitation
HCl	Hydrochloric acid
HCT	Heat coagulation time
Hz	Hertz
IgG	Immunoglobulin
K	Consistency index
Kg	Kilogram
kPa	Kilopascal

Lh ⁻¹	Liters per hour
Lm ⁻² h ⁻¹	Liter per meter square per hour
LMWP	Low molecular weight peptides
m	Meter
min	Minutes
mL	Milliliter
μL	Microliter
MPC	Milk protein concentrate
MT	Metric ton
n	Flow behavior index
NCN	Non-casein nitrogen
NF	Nanofiltration
NFDM	Non-fat dried milk
nm	Nanometer
NPN	Non-protein nitrogen
PF	Plate and frame filtration
RCT	Rennet coagulation time
s	Second
TP	Total protein

TS	Total solids
UF	Ultrafiltration
VCR	Volumetric concentration ratio
WP	Whey protein
α -LA	α -lactalbumin
α_{s1} -CN	α_{s1} casein
α_{s2} -CN	α_{s2} casein
β -CN	β -casein
β -LG	β -lactoglobulin
γ -CN	γ -casein
κ -CN	κ -casein

ABSTRACT

EVALUATION OF PROCESSING VARIABLES TO INCREASE THE SOLIDS OF
MILK PROTEIN CONCENTRATES PRIOR TO DRYING

ACHYUT MISHRA

2020

It is a common practice in dairy industries to nanofilter milk protein concentrate (MPC) to increase the solids and further drying efficiency. Increasing protein and solids in MPC increases viscosity and resist feed-flow in membrane filtration. Previous research proved that increased temperature and hydrodynamic cavitation (HC) have significantly reduced the viscosity of MPC which can potentially increase the level of solids by reducing concentration polarization during nanofiltration (NF). We utilized plate and frame filtration (PF) as an alternate method to reduce the viscosity of MPC.

In first objective, we studied the application of HC or elevated temperature (50°C) or both to concentrate MPC with 80% protein (MPC80) based on total solids (TS) using NF. Three replicates of MPC80 having 20% TS were concentrated in NF system at 22 and 50°C. HC did not have a significant impact on average flux but increased the TS significantly. Study showed that increased temperature or its combined action with HC improves NF performance in MPC filtration.

In second objective, we studied the functionality of MPC80 powder after spray drying of NF retentates from first objective. Study showed that, higher temperature NF impacted on rennet coagulation time (RCT), wetting time, and heat coagulation time of the powders. Both HC and elevated temperature did not impact on flowability and emulsion

capacity whereas HC alone improved dissolution and foaming capacity of the powders. Study showed that NF temperature and HC have important impacts on the functionality of MPC80.

In third objective, we studied the application of PF in MPC80 concentration at 22 and 50°C using flat-sheet ultrafiltration membrane. Average flux, final TS, and total protein to TS ratio were increased significantly at higher temperature PF. Study showed that increased temperature improves PF performance in MPC filtration.

In fourth objective, we studied the functionality of MPC powders after spray drying of feed and PF retentates from third objective. Higher temperature PF impacted on RCT, wettability and solubility but improved foaming capacity and emulsion stability of the powders. Study showed that temperature optimization is important for concentrating MPC80 in PF system to maintain its functionality.

Key words: milk protein concentrate, hydrodynamic cavitation, nanofiltration, plate and frame filtration, functionality

CHAPTER 1

Review of Literature

1.1 Milk protein concentrate and its market

Milk protein concentrate (**MPC**) is a complete protein that contains both casein (**CN**) and whey proteins (**WP**) in the same or similar ratio as found in skim milk (ADPI, 2016). In market, MPC are available based on their protein content, and the most common are MPC 42, MPC 70, MPC 80, MPC 85 (Patel, 2014). Because of having high protein and low level of lactose, MPC acts as an excellent ingredient mainly for cheese products, cultured products, dairy based beverages, pediatric nutrition, enteral foods, weight management products, powdered dietary supplements and sports nutrition products (ADPI, 2020). Because of its great functional properties, MPC also can be used in confectionaries, meat products, soup, ice-cream, and bakeries (Patel et al., 2014).

In the market, MPC is available mostly in the powder form. According to More (2020), the market of MPC is in increasing trend. The production of MPC was increased by 4.2% from 2012 (160,332 MT) to 2017 (188,469 MT). More (2020) also mentioned that, for the period of 2020 to 2025, the estimated compound annual growth rate would be 3.0%. According to U.S. Dairy Export Council (2020), export of MPC were up for the third straight year, finishing 5% above 2018. The market of MPC has been growing mainly in USA, Canada, Mexico, Europe, Brazil, Asia Pacific, and Africa (Data Intelo, 2019). The potential reasons of increasing market value might be due to its excellent functional role in variety of food products as well as people are getting more aware in their weight management by consuming quality protein (Patel et al., 2014).

1.2 MPC production and concentration

Mostly, MPC is produced by removing majority of water, lactose, and minerals by using ultrafiltration (**UF**) membrane. Higher protein contents in MPC, >65% based on total solids (**TS**), requires water diafiltration (**DF**) along with UF. However, from UF/DF process the TS in retentate will be limited to 20-22% (Marella, 2015). To concentrate further, MPC is processed either from evaporation or membrane process prior to drying. According to Jevons and Awe (2010), companies utilizing evaporation as a standard concentration step in the manufacture of dairy food ingredients can potentially reduce their carbon footprint with membrane technology. The cost of initial installation, further operation and maintenance for membrane process is lower when compared to other concentration technologies (Hilal et al., 2004). To get higher solids MPC prior to drying, industries follow either nanofiltration (**NF**) or reverse osmosis (**RO**) or evaporation. These days, industries have been preferring NF to concentrate MPC over the RO or traditional evaporation system. Past studies showed that, the functionality of MPC concentrated using membrane process is superior to evaporation because of high chances of protein denaturation during evaporation (Cao et al., 2015a, Rupp et al., 2018). During NF, monovalent ions can be diffused through NF membrane due to its looser membrane structure resulting higher volume reduction ratio at a similar transmembrane pressure when compared to RO, or NF can be performed at lower transmembrane pressure than RO to get same level of solids in the retentate (Meyer et al., 2016). Due to the lower concentration of monovalent ions, the quality of MPC powder from NF could be better in terms of solubility (Cao et al., 2015a) and heat stability (Syrios et al., 2011) when compared to RO.

1.3 Major hurdles in MPC production and concentration

1.3.1 Viscosity

During the production and further concentration of MPC, the viscosity increases with increasing the level of TS. In addition, when the ratio of total protein (TP) to total solids (TS) increase, the apparent viscosity increases considerably (Solanki and Rizvi, 2001), which can be considered as the primary hurdle in milk protein concentration using spiral wound membrane. In UF/DF system, DF helps in the reduction of viscosity to some extent during MPC production (Li et al., 2017). But in NF system, the viscosity continuously increased with increasing the level of solids and protein. Patel et al., (2009) reported that concentrates with higher protein content would increase their viscosity which may alter the functional properties of MPC after drying. The TS of MPC fluid can be increased by the application of heat during membrane filtration (Bastian et al., 1991), however the atomization during drying can be difficult due to its viscosity and that may impact on the properties of powder (Fryer, 1989, Bienvenue et al., 2003). Viscosity reduction not only helps in filtration performance but also helps in the reduction of energy cost during drying (Marella et al., 2015).

The viscosity of the milk concentrates is mainly depends on the heat treatments of the protein and the level of TS (Morison et al., 2013). Increased viscosity in heat-treated MPC is mostly caused by the aggregation of denatured WP on the surface of CN micelle; though, there are some other factors that can impact the viscosity of MPC including: TS, protein content, pH, availability of calcium chelators and their buffering capacity (Langley and Temple, 1985, Bienvenue et al., 2003, Anema et al., 2004, Considine et al., 2007, Anema et al., 2014). Karlsson et al., (2005) mentioned that the pH adjustment of

skim milk concentrates changes in the volume of CN micelles increased greatly and that impacts on the viscosity. When the pH decreased from 6.51 to 6.15, the viscosity of skim milk concentrate reduces, but if that goes below 6.15, the viscosity raises (Karlsson et al., 2005). Low temperature application in membrane filtration not only increases viscosity of the retentate but also increased concentration polarization and reduce the permeate flux (St-Gelais et al., 1992). Study conducted in skim milk concentration in UF showed that, the relative permeation flux was increased significantly when processed at 50°C compared when compared to 10°C (Méthot-Hains et al., 2016). They also mentioned that permeation flux decreased continuously though the temperature or transmembrane pressure altered because of the concentration polarization.

1.3.2 Concentration Polarization

When the filtration progresses during MPC concentration in membrane system, there is high chance of concentration polarization, which can be assumed as second major hurdle to be overcome in the development of a practical industrial unit operation specially for milk protein production industries (Porter, 1972). The Concentration polarization creates a large pressure drop within the module which reduces the filtration efficiency of the membrane when a viscous fluids like MPC is processed (Lipnizki et al., 2005). The increasing concentration of macromolecular solutes and colloidal species at the membrane surface starts gelling and forms a dynamic secondary membrane on top of the primary membrane module. This secondary membrane can offer the major resistance to flow (Porter, 1972, Lyster et al., 2010). The thickness of gel layer grows until the pressure-driven convective flow of solute with solvent toward the membrane surface equivalent to the concentration gradient-driven diffusive flow away from the surface

(Porter, 1972). Eventually, the local increase in concentration can lead to super saturation of sparingly soluble salts, which leads nucleation and causes the successive formation of a scaling layer on the membrane. When the nucleation occurs, the crystal grows and cover the membrane surface which reduces the active area for the permeation, results drastic reduction in the flux (Radu et al., 2014).

1.4 Hydrodynamic cavitation and its application to reduce the viscosity of MPC

Cavitation process is associated with the pressure and it happens when the local pressure goes below the vapor pressure relating to the temperature of liquid used in process. In the hydrodynamic cavitation (HC) of a fluid, cavitation occurs when fluid passed through a venturi like constricted device. In the constriction region, the fluid velocity increases due to the pressure drop and then form number of bubbles. However, in the downstream area of constriction, the recovery of pressure occurs because of the partial expansion and collapse of cavitated bubbles results release of high amount of energy in terms of rising temperature and pressure (Franc and Michel, 2006). The temperature and pressure generated locally can modify the properties of macromolecules (Gogate and Pandit, 2005). In addition, during cavitation, some highly reactive free radicals such as hydroxyls, hydrogen peroxides, etc. are generated and can be helpful in the degradation of contaminants (Gogate, 2011b) and destruction of microorganisms.

Milk protein properties can be modified by number of mechanical methods such as HC, extrusion porosification, ultrasonication, microfluidisation, or high shear treatment of the milk (Gogate and Pandit, 2005, Li et al., 2018) have been reported which can improve the viscosity of the milk concentrate or solubility of the powder after drying. Among the method, HC has more advantages. It can be applied to a large volume and

exhibits rapid recovery of pressure. The shockwave produced due to the collapse of numerous cavitated bubbles during the recovery of pressure creates an efficient and microscopic mixing (Tao et al., 2016, Oestergaard, 2015), and the friction of rotor and liquid generates scale-free heating (Gogate, 2011a). Oestergaard (2015) observed a reduction of viscosity by 20% when HC was applied on whey protein concentrate containing 80% protein. In the same paper, it is also mentioned that, HC can work for the high viscosity caseinate product and improves the level of solids >15% before drying. Ultrasonication and HC has similar mechanisms in changing structural mechanism, however, HC is considered more energy efficient. The use of HC significantly reduced the viscosity of MPC prior to drying and improved the efficiency of spray dryer (Li et al., 2018), which can also be applicable to the manufacture of infant formula. Similarly, cavitation can be applied between ultrafiltration (UF) and nanofiltration (NF) to ensure low viscosity of the feed material for improving the efficiency of NF. Efficient NF may results higher solids level in the retentate which can replace the evaporation stage and saves the functionality of protein (Li et al., 2018, Rupp et al., 2018).

1.5 Application of elevated temperature to reduce the viscosity of MPC

In membrane process, operating conditions can impact both permeate flux and solute rejection, where permeate flux is very sensitive to feed temperature, which can be explained by equation i.

$$J = J_0 \left(\frac{\mu_0}{\mu} \right) \quad [i]$$

Where, J is the permeate flux through the membrane, μ is the feed viscosity at desired temperature, J_0 is the permeate flux at a reference temperature, and μ_0 is the viscosity of

feed at reference temperature. J_0 and μ_0 are constants and are normally defined by the membrane manufacturer mostly at room temperature. From the equation 1, it can be proved that, there is the inverse relation between permeate flux and feed viscosity at fixed temperature, however the rate might not be linear (Kowalska et al., 2004). Permeate flux increases as the feed temperature increases however might be detected for instance as a deviation in the linear increase of flux with increasing temperature. A past experiment in UF made of polyethersulphone (PES) membranes showed a non-linear increase of flux with increasing temperature in the range of 25-55°C (Kowalska et al., 2004), and they further explained that the non-linear flux increase was credited to the thermal expansion of the membrane. Membrane filtration favors by lowering liquid viscosity which supports to increase mass transport across the membrane (Marcelo and Rizvi 2008). High temperature decreases the feed viscosity due to an increase in the feed temperature which eventually improves the permeate flux (Baker, 2004, Jönsson et al., 1990). High-temperature also decrease the density of the process fluid and an increase the diffusivity of the fluid contents (Jönsson et al., 1990, Cheryan, 1998). The cost of pumping fluid can be cheaper at a higher temperature due to the reduction of fluid viscosity (Cheryan, 1998). At higher temperature NF, the concentration polarization also can be reduced (Mänttari et al., 2002).

1.6 Importance of MPC

1.6.1. Nutritional importance

Protein is the major nutrition provided by MPC. The protein available in MPC is a complete protein. Among proteins, it contains micellar CN, WP, and bioactive proteins in the same ratio as in milk (Sindayikengera and Xia, 2006). Among these proteins, WP is

very useful for targeting daily protein goals during weight management practices because of its high absorption nature. The benefits of WP include muscle gain in combination with resistance training, limiting muscle loss during low-calorie diets, and limits fat gain to some extent in the period of high calorie intake (Pennings et al., 2011). As the protein content of MPC increases, the level of lactose decrease. Protein-bound minerals mainly Ca and P, which generally do not pass through the membrane are the major minerals available in the MPC (O’Kennedy, 2009). This high-protein low-lactose ratio makes MPC an excellent ingredient for protein-fortified beverages and foods, low-carbohydrate foods such as nonfat yogurt, ice cream, and cheese (ADPI, 2016, Mistry, 2002). Specialized health products production from the milk protein ingredients based upon the claims of anti-oxidative, antihypertensive, immunomodulating, antimicrobial, antiulcerogenic, and anti-inflammatory properties have been reported (Meisel and Schlimme, 1996, Darewicz et al., 2006, Eriksen et al., 2008, Tsopmo et al., 2011, Martínez-Maqueda et al., 2012). High protein low lactose MPC also can be used in the food formulation focusing dietetic patients (Augustin et al., 2011).

1.6.2. Functional importance

Milk proteins have several functional properties which provide desirable attributes to the final food and dairy products mainly for water holding, gelation, foaming, etc. (Singh, 2011) (Singh, 2011). In MPC, casein is the dominant protein and can hold large amount of water. One-gram casein protein can hold approximately four-gram water (McMahon and Oommen 2013), which shows MPC has high water holding capacity. Gelation is directly related to the dispersibility of MPC and mostly utilized in dairy based beverages (Kuo and Harper, 2003). Milk protein concentrate has wider applications in food products

that require greater functionality such as confectionery and bakery products, infant formulations, meat based products, soups and sauces, dry seasoning blends, and cheese base for soft cheese manufacturing (Kelly and Kelly, 1995). Because of high water absorption property of casein, MPC shows good gelling or thickening activity, so it can be utilized in the preparation of various food products where the bulkiness is essential (Patel et al., 2014). Application of MPC in cakes, whipped toppings etc. gives good results in terms of foaming where milk proteins provide desirable air–water dispersions in such type of products and provides very stable films of bubbles (Uluko et al., 2016). Foaming capacity is related to the surface-active properties of proteins available in MPC powders (Huppertz, 2010, Ye, 2011). The functionality of MPC powder depends on the processing factors (for e.g. microwave, high pressure, ultrasound, etc.) and drying conditions (Chen and Patel, 2008). Most of the functional properties of proteins vary with pH, temperature, concentration of calcium ions, lactose, and hydrocolloids (Singh, 2011). Similarly, high temperature and acidic or basic pH also leads protein to protein interactions and alters the functionality of milk protein (Chandrapala et al., 2015). During food formulation, milk proteins also employ several interdependent functional properties when it is used as an ingredient and that also depends on the food matrix (Singh, 2011).

1.7 Membrane filtration

Application of membrane in food processing is highly demanding for the separation, concentration, and desalination (Spillman et al., 2007, Uragami, 2010, Panagopoulos et al., 2019). The most common membrane techniques are UF, microfiltration, NF, RO and dialysis (Baker, 2000, Uragami et al., 2013). The advantage of membrane process in food processing is that there is very less changes in the nutritional and functional

characteristics of the product. Membranes are differed by their pore size in terms of molecular cut-off, in which >90% of the targeted molecules can be retained in the retentate (Singh, 2005). In milk processing, membranes are used to separate milk components to produce milk protein concentrates and isolates, whey protein concentrates and isolates, lactose separation from whey, dairy minerals, milk casein concentrates, etc. (Kumar et al., 2013). Membrane technology has been utilizing in place of many important processing stages of milk in dairy industry such as centrifugation, bactofugation, evaporation, demineralization of whey, etc. (Pouliot, 2008b). Membranes such as NF, RO and UF are mostly made from polymer and membranes for microfiltration is made from ceramic (Kumar et al., 2013, Lee et al., 2015). Membrane processes have been widely used to develop and manufacture dairy-based proteins ingredients from milk or whey. Membranes such as MF, UF, NF and RO membranes are, recognized as the pressure-driven membrane process in which the main driving force for separation of these processes is transmembrane pressure, characterized by pore sizes mostly between 0.1 nm and 0.1 μm by operating pressures between 0.01 and 5 MPa (Cheryan, 1998, Mulder, 2012). The operating pressures are based on the pore size. NF and RO membranes require more operating pressure compare to MF and UF. Some UF membranes and most of the NF membranes are electrically charged where electrostatic interactions and Donnan effects applied in their separation systems (Pouliot, 2008b). The separation principle of UF and NF is not only based on the pore sizes but also in the charge of the molecules/solutes and their affinity for the filtering membrane are also important (Kelly and Kelly, 1995). The major limitation of membranes in spiral wound

form has the limitation in generating of large pressure drop within the module when possessing highly viscous fluids (Lipnizki et al., 2005).

1.7.1 Ultrafiltration

Membrane having pore size between 1 to 500 nm (1-200 kDa molecular weight cut-off) are categorized as UF membrane. The separation mechanisms of UF is sieving or charge or both and the operating pressure required 0.1 to 1.0 MPa (Cheryan, 1998, Mulder, 2012). UF can be fabricated in number of modules mainly tubular, hollow fiber, spiral wound and plate and frame (Cheryan, 1998). The UF separates mainly soluble proteins, caseins, and macropeptides and the most common commercial ingredients such as WP concentrate, β -LG, α -LA, and MPC are already popular in the market (Pouliot, 2008a). With the advancement of superior quality membranes, UF has become a most important separation process in the dairy industry. UF rejects proteins and fat and allows lactose, minerals and water of skim milk (van Reis and Zydney, 2007). There are variety of UF modules in which hydrostatic pressure forces a liquid against a semi-permeable membrane. Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane (Nath, 2008). When milk is filtered using UF, it retains the fat globules and the proteins whereas majority of inorganic salts and lactose, along with water, are removed as permeate. The retentate is a partially concentrated milk with low level of lactose and minerals. The concentration ratio is generally 3 to 5-fold. If further reduction of lactose and mineral is required, the retentate is further diluted with water and ultrafiltered again, which is known as DF (Berk, 2013). Application of UF are mainly focused on pre-concentration of milk for cheese production and manufacture of whey protein concentrates and milk protein

concentrates (Cheryan, 1998, Berk, 2013). In cheese production, UF helps in increasing yield, nutritional value mainly protein, making easier standardization of the solids and reducing rennet dosing (Mistry, 2003). Ultrafiltration has been widely used in dairy industries to produce a number of dairy ingredients such as MPC (Jimenez-Flores and Kosikowski, 1986, Patel et al., 1991, Mistry, 2002) and protein concentrates from cheese whey (Marella, 2009) and, whey protein fractions (Muller et al., 2003, Mehra and Kelly, 2004, Marella et al., 2011).

1.7.2 Nanofiltration

The NF membrane is a membrane with properties between those of RO and UF membranes (Cao et al., 2015b). The development of NF dates to the 1970s when RO membranes were modified to get a better water flux at relatively low pressures were developed. The pore size of NF membrane is between 0.1 to 1 nm (200-1000 Da molecular weight cut-off) are categorized as NF membrane. NF separates the material on the basis of sieving or charge or both and it can be operated with the pressure between 1.5 to 3 MPa (Cheryan, 1998, Mulder, 2012). NF is utilized for partial demineralization and, at the same time, increases the TS in the retentate so that further thermal evaporation is not required. In general, NF permits monovalent salts to pass through while it holds multivalent salts (Kelly and Kelly, 1995). NF also can be available in several modules, for e.g. tubular, hollow fiber, spiral wound, plate and frame etc. (Cheryan, 1998). The current common application of NF in the dairy industry is for concentrating and desalinating whey (Suárez et al., 2006, Cuartasuribe et al., 2007). The advantages of NF includes lower initial investment, lower operation and maintenance costs, lower operation pressure, higher flux, and higher retention of multivalent salts and organic molecules

when compared to RO (Hilal et al., 2004). The NF separates mainly indigenous peptides, and divalent cations. The most common commercial ingredients such as Bioactive milk, WP hydrolysate, glycomacropeptide are mostly produced by using NF (Pouliot, 2008a). Generally, NF allows lower temperatures, the milk protein would be less exposed to heat treatment, which may results less denaturation and have better functionality when compared to evaporation (Cao et al., 2015a). However, the NF system also has the limitation of operating viscous liquids such as milk protein concentrates, which is due to the high water binding properties of casein micelles (Lipnizki et al., 2005, McMahon and Oommen, 2013). Similarly, the degree of interaction between the casein micelles go higher and forms hairy layers from the overlapping of micelles with increasing the level of solids, results very high viscosity and difficult to flow (Bouchoux et al., 2009, Dahbi et al., 2010).

1.8 Energy consumption in membrane process

The key parameter in the selection of any processing system is the energy efficiency of that system (Humphrey, 1997), however the exact estimation might be difficult because of its multifaceted concept (Kondepudi and Prigogine, 2014). The energy efficiency of membrane operations is relatively high because they do not require a phase change (Koros and Lively, 2012). The total energy consumed for a typical UF system in milk filtration ranged from 50.08 to 62.54 kJL⁻¹ of retentate, or 18.18 to 21.65 kJL⁻¹ of permeate. Similarly, the energy consumed for cleaning can be in the range of 87 to 107 kJ at different operational pressures (Shenana et al., 2010). In membrane process, when milk is concentrated using UF or NF system, the concentrate becomes viscous when it starts gaining solids and requires more energy to pump. The energy needed to concentrate milk

by UF, or NF or UF in flat sheet module (e.g. PF) is mainly ascribed to the pumping (Ghidossi et al., 2006). In these filtration systems, there would be at least two pumps; feeding pump and recirculation pump. The feed pump power and recirculation pump power can be calculated using equation ii and equation iii, respectively.

$$W_{\text{feed}} = JA_m P_2 \quad [\text{ii}]$$

$$W_{\text{rec}} = Q_{\text{rec}}(P_1 - P_2) \quad [\text{iii}]$$

Where J is the permeate flux, A_m is the membrane surface area, Q_{rec} is the average circulation flow rate, and P_1 and P_2 are inlet and outlet pressure across the membrane. Now, total power can be calculated by using equation v.

$$W_{\text{total}} = W_{\text{feed}} + W_{\text{rec}} \quad [\text{v}]$$

Similarly, the energy consumed per unit volume of permeate (kJm^{-3}) produced is calculated as in equation v, Where, Z is the pumping efficiency.

$$E_c = \frac{W_{\text{total}}}{JA_m Z} \quad [\text{v}]$$

To enable a practical comparison of the different membrane systems, the specific energy requirement is to be calculated. Considering that the practical energy demand of the pumps is 80-90% of the ideal capacity (CIPEC, 2002), the specific energy requirement of the membrane process E_m in kJkg^{-1} can be calculated using equation vi as described by (Koros and Lively, 2012).

$$E_m = 0.8(E_F + E_R)m_{\text{Per}} \quad [\text{vi}]$$

Where E_F and E_R are the capacity claimed for the feed and recirculation pumps in kJ , respectively, and m_{Per} is the mass of permeate in kg . The specific primary energy

demand $E_{m,p}$ in kJkg^{-1} . The real energy requirement of the separation process (W_R) is compared to the minimum work (W_M), so that the energy efficiency of the process ranges between 0 and 1. The value of W_R depends on the operating conditions, performance of equipment and process design, and can be obtained from experiments or industrial plant data through energy balances (Castel and Favre, 2018). In most of the membrane process the efficiency of the power consumption is considered as 35% (Vermaas et al., 2011). As an alternate approach, power consumption can also be calculated from inline voltage (V) and amperage (I) measurements method (Gavazzi-April et al., 2018) as in equation vii.

$$W = \sqrt{3} \cos\theta \times V \times I \quad [\text{vii}]$$

Where, $\cos\theta$ is a power factor which is dependent on the technical specification of the UF or NF or PF system. From equation vii, we can also calculate the power consumed by the HC motor. The energy efficiency of high-pressure displacement pump used in HC can be in the range of 20-40% and 50-70% when the flowrate adjusted $<10 \text{ m}^3\text{h}^{-1}$ and $>10 \text{ m}^3\text{h}^{-1}$, respectively (Shah et al., 1999).

1.9 Spray drying of MPC

Spray drying is a multiphase and complex drying process with number of interactions between droplets, air, and particle of different flow trajectories (Fu et al., 2012). During drying of milk products, viscosity and surface tension become extremely high around a critical water activity level which is dependent on composition and temperature (Adhikari et al., 2007, Palzer, 2007, O'Callaghan, 2010). Protein rich milk products have sticky and rubbery states around a point where they become clogged due to concentration. It is fact that colloidal glass transitions can be observed in a concentrated and stabilised colloidal system (Weeks et al., 2000). However, at later stages of drying

the stickiness of protein-containing dispersions tends to be dominated by the carbohydrate components (Adhikari et al., 2009). The high viscosity and or high surface tension state is sometimes referred to as a rubbery state (Roos and Karel, 1992). There is a region roughly between the rubbery and non-sticky states known as a glass transition region. Stickiness problems in spray drying are related to the powder encountering equipment surfaces whilst in the glass transition state, sometimes referred to as a plastic state (Brostow et al., 2008).

The spray drying chamber is engineered for progressive removal of water, simultaneous with decreasing air temperature and increasing air humidity as the drying air takes up moisture. In most of cases, the drying chamber is a vertical tower made up of cylindrical or conical sections and, ideally, the states of powder and air should be symmetrical with respect to the centre line, i.e. axial symmetry should prevail. If such asymmetry extends beyond the immediate atomisation zone, it needs to be addressed as a design issue involving the air distribution system at the point of entry (O'Callaghan and Hogan, 2013). It is believed that particle size plays a role in stickiness, with smaller particles being more prone to stickiness (Adhikari et al., 2001) but not much experimental work has been cited on this issue. It has been shown that inlet and outlet drying temperatures and feed solids level influence the surface composition of spray-dried powders (Kim et al., 2009). Central swirling of hot air is assumed steady flow in spray drying. A recent review suggests that the airflow pattern, specifically the central jet, has tendency to exhibit self-sustained oscillatory behavior. There is a big difference between the wall deposition rates and locations for steady and unsteady swirling flows. While data on pilot size spray dryers are more realistically obtained for model validation,

significant uncertainty remains when the model is to be applied to full-scale dryers (Woo and Bhandari, 2013).

For the prediction of the drying behavior of dairy products, a lot of fundamental studies are available committed to predicting the spray drying behavior of dairy powders (Woo and Bhandari, 2013). The drying kinetics is important to predict the size and determine the operating parameters required for drying dairy powders. Typical in spray drying is the presence of a constant drying period in the initial condition when the droplet is still 'saturated' with water and the falling rate period in which the drying rate deteriorates due to reduction of surface moisture (Lin and Chen, 2007). Dimpler and Kulozik (2015) described that the MPC and nanovesicle mixtures were spray dried at 160°C inlet and 75°C outlet air temperature. DeCastro and Harper (2001) found no difference in the quality when MPC was dried at an outlet temperature range 65 to 90 °C in terms of protein denaturation, but the moisture content and rehydration rate of the resulting particles was differed. When high milk protein powders are dried, fat and protein located on the surface of the particle whereas lactose remains in the core (Kim et al., 2003, Gaiani et al., 2010). Similarly, at lower outlet drying temperature might increase fat and protein content on the surface composition of milk powder which impacts on the functionality of the powder (Gaiani et al., 2010). Drying parameters also affect the particle morphological characters of MPC powders including size, shape, porosity, and surface wrinkles and the characters might also influence by inlet and outlet drying temperatures, and type of atomizers (Maa et al., 1997). Functional properties are greatly impacted by spray drying conditions (Thomas et al., 2004, Chen and Patel, 2008),

and directly influence on wetting and rehydration properties and solubility (Gaiani et al., 2007, Fang et al., 2011, Fang et al., 2012).

1.10 Functionality of MPC powders

Functional properties of milk powders are generally attributed to milk proteins and their interactions (Sikand et al., 2011, Uluko et al., 2016, Rupp et al., 2018). Interfacial properties of milk proteins seem very important for the use of milk powders as food ingredients (Thomas et al., 2004, Rouimi et al., 2005). Most of the functional properties of MPC powders depends on the filtration and concentration conditions, drying conditions, storage time and temperatures (Anema et al., 2006, Havea, 2006, Singh, 2007, Rupp et al., 2018). In comparison to WP concentrate, MPC has poor functional properties (Singh and Ye, 2020) and needs additional studies from which the findings help to improve the functionality of MPC and increase market value. Some of the important functional properties have been discussed below.

1.10.1 Wettability

There is a four steps process in reconstituting of dairy powder such as wetting, submerging, dispersing, and dissolving (Freudig et al., 1999). Among these, wetting initiates the rehydration and mostly depends on the particle size and surface composition. Dairy powders having larger particle size show faster wetting due to having larger pores (Singh and Newstead, 1992). The viscosity of concentrate prior to spray drying and size of nozzle influences on the droplet size distribution and thereby particle size of powder (Andersen, 1986). So agglomeration of particles to make larger units and adding surfactant for e.g. lecithin to the powders are commonly used to improve the wetting properties of dairy based powders (Kim et al., 2002). The surface composition can be

altered during ageing of powder. Gaiani et. al., (2007) mentioned that during storage of dairy powder, the lipid coverage on the surface can be changed which might reduce the wettability of the powder particle. Fat renders the powder surface hydrophobic with a large contact angle between the powder and penetrating water which delays wetting (Granelli et al., 1996). So, the migration of components on the surface of powder during drying and aging changes the surface composition thereby alters the wetting property of the powder (Kim et al., 2009). The wettability of powders has been assessed using different techniques such as, International Dairy Federation (IDF 1979), dynamic contact angle measurement (Dupas, 2012) and the turbidity method (Gaiani et al., 2009a). A slimy skin is formed at the powder-water interface impeded water penetration and contributes to poor wetting (Fitzpatrick et al., 2017). High shear mixing improves wetting for poor wetting powders. Temperature impacts on wetting powder to powder. High temperature reduced the wetting of milk protein isolate and caseinate powders because of forming stronger films and interfere in water penetrating, however chocolate and high fat powders can be wetted faster at higher temperature water (Fitzpatrick et al., 2017).

1.10.2 Dissolution characteristics and solubility

Solubility is the most important functional parameter to characterize MPC powders (Sikand et al., 2011). The dissolution characteristics of MPC powder are mostly depends on its processing condition, powder composition, storage and dissolution conditions (Anema et al., 2006, Hauser and Amamcharla, 2016). During MPC production, number of thermal processing steps leads to the aggregation of caseins and whey proteins, denaturation of whey proteins and forming protein complex may lead to the decrease in solubility of MPC powders (Fang et al., 2011, Babu and Amamcharla, 2018). The major

factors of solubility of MPC powder during reconstitution are temperature and flow velocity of water during reconstitution (Pierre et al., 1992, Schuck et al., 1994). Higher viscosity arising from increased TS in the concentrates reduces the solubility of the powder after spray drying (Bloore et al., 1981). The rehydration rate of MPC powders containing >80% protein is low due to poor dispersible casein fractions and the rehydration rate further decreased with increasing storage time and temperature (Mimouni et al., 2010, Crowley et al., 2015b). The poor rehydration behavior of casein rich powders might be due to slow movement of water into primary particles (Richard et al., 2013). Milk powders having fragments of CN micelles can prejudice to further protein-protein interactions during spray drying and lower the functionality of the powder, mainly in rehydration (Singh, 2007). The maximum solubility of MPC powders can be found at fresh right after production and the solubility reduces with time and the reduction of solubility is more severe at high temperature storage (Fang et al., 2012, Udabage et al., 2012, Huppertz et al., 2017). When MPC stored at higher relative humidity, proteins undergo conformational modifications and interaction of protein and water, results poor insolubility (Haque et al., 2011). Various methods have been developed to test the dissolution characteristics of MPC including centrifugation and fractionation, static light scattering microscopy, nuclear magnetic resonance, focused beam reflectance measurement (FBRM), etc. (Mimouni et al., 2009, Fang et al., 2011, Haque et al., 2012, Le et al., 2012).

Insoluble materials sedimented at the bottom of tube after centrifugation determines the solubility index, higher the sedimentation lower the solubility index (Anema et al., 2006, Havea, 2006). Babu and Amamcharla, (2018) described that front-face

fluorescence spectroscopy combined with partial least squares regression can be utilized as a nondestructive technique to predict the solubility and dissolution characteristics of MPC powders based on fluorescence spectra of tryptophan and Maillard products in the powder. The steps of dissolution in water such as wetting, sinking and dispersing directly related to the solubility (Thomas et al., 2004; Schuck, 2011). The solubility of MPC in water at low temperature is limited but it increases with increasing temperature (Mistry and Hassan, 1991, Schuck et al., 2002, Baldwin et al., 2007). The solubility MPC also depends on the protein content. Higher the protein, the powder is less soluble (Sikand et al., 2011). Researchers have correlated rehydration characteristics of powders with particle size (Beliciu and Moraru, 2009), viscosity (Gaiani et al., 2006), turbidity (Gaiani et al., 2009a). In the phase of rehydration, dissolution of minerals from powder to water determines the rehydration rate (Augustin and Clarke, 1991). Solubility of MPC powder can be enhanced by number of techniques such as static high pressure, high shear treatment, ultrasound, adding NaCl etc. (Augustin et al., 2012, Mao et al., 2012, Udabage et al., 2012). MPC concentration by evaporation might impact on the solubility due to changes in casein colloid (Singh, 2007), but the NF has no or very little effect on the solubility because of low processing temperature (Cao et al., 2015b). The solubility might also differ with the drying scale such as laboratory and industrial and the powder particles from small scale dryers contains more protein on the surface and having more collapsed structure (Fyfe et al., 2011) which may leads poor rehydration.

1.10.3 Emulsifying capacity and emulsion stability

Milk proteins have gained much interest for its emulsifying properties either as a whole or its fractions such as whey proteins, α -LA, β -LG, and bovine serum albumin

because of their surface active properties (Dickinson, 1998, Brun and Dalgleish, 1999, McCrae et al., 1999, Kim et al., 2005a). Milk protein plays a vital role in number of food products such as market milk, cream, coffee creamer, protein beverages, desserts, ice-cream, etc. in homogenous distribution of fat and acts as an emulsifier (Dickinson, 1998, Singh, 2011). The emulsifying capacity is defined as the maximum capacity of oil holding by an emulsifier that can be dispersed in a protein solution before phase inversion (Euston et al., 1995) while emulsion stability relates to the time before phase inversion (Thomas et al., 2004). In emulsion forming process, milk proteins adsorb at the interface or at the surface of the oil droplets and form a thin layer. Denatured caseins and casein fractions are considered more flexible and faster than whey proteins to form emulsion (Singh and Ye, 2013). WP undergoes cross-linking during heating. When heat is gently applied, the emulsions form soft solids and attributed to good mouthfeel (Mantovani et al., 2016). WP are heat sensitive and majority of WP denatures at 75 to 80°C (Millqvist-Fureby et al., 2001). Casein micelles or stabilized emulsion of caseinates are mostly heat stable and the factor for determining emulsion stability is the WP (Livney et al., 2003). Emulsifying properties of MPC can be impacted by types of oil used, protein concentration and homogenization process (Horn et al., 2012). In comparison to MPC, WP concentrate and sodium caseinate show better emulsifying ability at relatively low protein to oil ratio (Ye and Singh, 2001). The emulsifying capacity can be improved by using Ca reduced MPC and the stability can be improved by using high protein MPC (Ye, 2011). More aggregated proteins, in general, give emulsions with a higher surface coverage, a higher surface viscosity and greater adsorbed layer dimensions, and so might be expected to have a greater stabilizing effect on the emulsion droplets (Euston and

Hirst, 2000). High spray drying temperature allows less proteins to migrate towards the surface which impacts on the surface properties of the milk powder (Nijdam and Langrish, 2006) which might impacts on the emulsion properties.

1.10.4 Foaming capacity and foam stability

Foams consist of a discrete gas or bubble phase dispersed in either liquid or solid continuous phase (Mahmood et al., 2014). Milk proteins play an important role in foam forming and stabilizing foams in aerated dairy products such as ice-cream, cakes, whipped toppings, etc. (Dickinson, 2003, Huppertz, 2010). Foaming behavior of milk proteins depends on heat treatment, pH, and ionic strength (Tolstoguzov et al., 1997, Tcholakova et al., 2006, Marinova et al., 2009). Heating globular protein such as WP to achieve partial denaturation increase the foaming properties. Heating helps in unfolding protein globules and exposes hydrophobic sites which may adsorb immediately to air-water interfaces and lower interfacial tension, the condition is favorable to trap more and more air (Meena et al., 2017). However, extensive denaturation of WP due to heat may reduce the ability of protein to form foams (Mauer, 2003). Application of ethylenediaminetetraacetic acid dispersed milk proteins efficiently by dissociating casein micelles and improves foaming properties (Ward et al., 1997, Zhang and Goff, 2004). Proteins stay in the interface of a film around the particles during foaming and impart the desired kinetic stability to the dispersions such as emulsion and foaming (Rodríguez Patino et al., 2008). The capacity to stabilize air-water interface to create a foam is known as foaming capacity which can be calculated as the percentage increase of the volume after whipping on the basis of initial volume, and also known as overrun (Yankov and Panchev, 1996, Singh, 2011). Foaming capacity also can be evaluated by measuring the

electrical conductivity of the foam (Thomas et al., 2004). Foam stability is defined as the time required to lose either 50% of the liquid or 50% of the volume of the foam (Mauer, 2003). Foaming capacity increases with increasing protein solubility. In comparison to WP concentrate, MPC is poorer in both foaming capacity and stability (Singh, 2011).

1.10.5 Rennet coagulation time

When rennet is added in the milk, the casein micelles flocculate due to losing hydrophilic domain from κ -CN which further goes to form casein aggregates, a three dimensional network of protein gel (Horne and Banks, 2004, Muñoz et al., 2017). The formation of the aggregates in the renneted milk is strongly dependent on the temperature and rennet concentration (Muñoz et al., 2017). The increased concentration of protein and high incubation temperature increase gel firming rate and gel firmness (Guinee et al., 1996, Upreti et al., 2011, Panthi et al., 2019). The pH and ionic calcium concentration are also crucial in gel forming activity (Nájera et al., 2003, Mishra et al., 2005). The coagulation properties of a renneted gel are generally described by the storage modulus (G') and loss modulus (G'') from rheological test, which can be utilized to calculate rennet coagulation time (**RCT**) (Sandra et al., 2011). Rennet-induced gels entrap fat and whey in the available protein network (Ong et al., 2013). Usually, the coagulation temperature in cheese manufacturing is generally set between 20 to 36°C (Panthi et al., 2019). The coagulation temperature and protein concentration in renneted milk determines the microstructure and syneresis of the coagulum during cheesemaking (McMahon and Brown, 1982, Van Vliet et al., 1991, Lu et al., 2017). RCT of a renneted milk can be estimated using rheological method which is equivalent to the time in minute when the storage modulus becomes 1 Pa in a strain sweep test (Lucey, 2002; Zoon et al.,

1988), but in a similar test, Lu et al., (2017) determined RCT when the storage modulus equaled to the loss modulus. In cheese making, when the concentration in recombined milk increase, the RCT decrease and curd firming increase (Sharma et al., 1993, Orme, 2000). The increased concentration of ionic calcium for e.g. CaCl_2 in the cheese milk shorten the RCT. Past studies suggested that the renneting as well as physicochemical properties of casein micelles are affected when concentrating skim milk by UF (Ferrer et al., 2011). When a protein rich cheese milk is prepared using low temperature UF, the buffering capacity of the milk reduced due to higher loss of soluble calcium and phosphorus (St-Gelais et al., 1992). When DF is applied during the production of protein rich cheese milk, the dilution of the serum phase with water at the time of DF affects the integrity of casein micelles and loss of soluble Ca occurs which eventually increased the RCT (Li and Corredig, 2014). Past study showed that, for the skim milk ready for the cheese preparation, a 1mM addition of CaCl_2 showed best renneting action without affecting in gelation mechanism (Sandra et al., 2012).

1.10.6 Heat stability

Milk powders are reconstituted and subjected to heat treatments during the formulation of number of food products. Dairy processors and researchers have been taking interest in heat stability test of milk and milk products when sterilization and evaporation became demanding techniques for 100 years (Singh, 2004). Caseins have very good heat stability because of the absence of secondary and tertiary structure and presence of complex quaternary structure, whereas non-casein proteins in milk are more susceptible to heat denaturation and denatured completely upon heating at 90° C for ≥ 10 min (Fox and Morrissey, 1977). During processing, the complexing of whey and casein proteins in milk

or milk protein solutions determine its heat stability (Oldfield et al., 2000). Heat stability of milk is greatly influenced by β -LG as it is the most heat sensitive of among the milk protein fractions. β -LG to κ -CN ratio influences the heat stability of milk to a large extent (Tessier and Rose, 1964, Gazi and Huppertz, 2015). The heat stability is the ability of concentrated milk systems to withstand a certain heat load, and it is important when the reconstituted milk requires high temperature processing such as ultra-high temperature processing, canning etc. to retain the product quality from heat-induced destabilization (Crowley et al., 2014b). Thermal processing may raise the production of organic acids due to bacterial growth also reduce the heat stability of the final product (Fox, 1981, O'Brien, 2009). Heat stability of milk or protein rich dairy ingredients is based on its compositional factors (Horne and Muir, 1990). Heat stability of reconstituted MPC is weaker when increases the concentration of protein (Crowley et al., 2014b). Increasing ionic calcium activity also shows the negative impact on the heat stability (Philippe et al., 2003, Sievanen et al., 2008) having shorter heat coagulation time (**HCT**). The HCT of reconstituted milk decreases with decreasing pH and increasing ionic strength mainly due to calcium ions (Sievanen et al., 2008, On-Nom et al., 2012, Crowley et al., 2015a). Crowley et al. (2014) mentioned that reconstituted MPC powders at 3.5% protein (wt/wt) had decreased HCT as the protein content of the powders increased from 35 to 90% (w/w, dry basis), and claimed that the reduced HCT was due to a higher level of ionic calcium in dispersions prepared from powders having higher protein level.

1.10.7 Whey protein and denatured protein

It is considered that MPC contains CN and WP in similar ratio like skim milk (O'Kennedy, 2009). Among the total proteins in milk, WP constitute 20% including β -

LG ($\sim 3.2 \text{ gL}^{-1}$), α -LA ($\sim 1.2 \text{ gL}^{-1}$), bovine serum albumin ($\sim 0.4 \text{ gL}^{-1}$), and immunoglobulins ($\sim 0.7 \text{ gL}^{-1}$) (Raikos, 2010). When milk is exposed to thermal processing, whey proteins may undergo structural changes such as denaturation and extent of denaturation depends on the heating conditions (Raikos, 2010). The denaturation of WP by heat is a major issue in terms of processing as well as quality in the dairy industry. Denaturation of whey proteins is come with by releasing sulfur-containing compounds such as hydrogen sulfide and methanethiol which give typical cooked flavors in heated milk (Al-Attabi and others 2009). Aggregation of WP involves the interaction of a free -SH group with the S-S bond of cystine-containing proteins such as β -LG, κ -CN, α -LA, and bovine serum albumin via -SH and S-S switching reactions (Considine et al., 2007). The hydrophobic groups initiate the formation of hydrophobic links between β -LG and α -LA and form aggregates when heated at increased temperatures ($>70^\circ\text{C}$) (Oldfield et al., 1998). The extent of protein denaturation and aggregation is affected by heating conditions, pH and ionic strength, and goes in subsequent interactions with the casein micelles which may increase the size of micelle (Anema et al., 2003). This protein to protein interactions undergo irreversible aggregation of proteins for forming protein complexes of different molecular weights based on heating condition and the protein composition. When milk is heated at its normal pH, the WP reacts primarily with the κ -CN on the casein micelles (Donato et al., 2007). Gautam (1994) described that, high temperature membrane filtration of milk also accelerates membrane-fouling due to the gelling effect caused by protein denaturation. The change of the casein micelle size induced by different heating conditions in centrifuged skim milk was reported by Ono et al., (1999).

1.10.8 Rheological properties

Rheological properties of dairy ingredients are very important and have high significance for food formulation for the selection and the design of processing units such as pumping, mixing, heating, and cooling (Sauer et al., 2012). Rheological information also helps in predicting sensorial attribute of the finished product such as mouthfeel (Hermansson, 1975). In past research, freeze-concentrated skim milk up to 25% solids showed Newtonian behavior (Chang and Hartel, 1997) and evaporated milk at solids greater than 22.3% showed non-Newtonian behavior (Vélez-Ruiz et al., 1997). These differences can be due to the differences in composition of the concentrates from respective systems. It was claimed that the differences in flow properties of different types of concentrated milks are a function of both the type and quantity of protein retained (Solanki and Rizvi, 2001). According to Sauer et al. (2012), micellar casein concentrate showed shear-thinning behavior when the casein concentration increased more than 7.5% with increasing viscosity, however the viscosity was decreased when measured at elevated temperature. They also mentioned that the level of serum protein played crucial role in the viscosity of the micellar casein concentrate dispersion. O'Donnell and Butler (1999) observed the flow behavior of 20-26% MPC85 solution showed shear thinning behavior and the effect of shear was sufficiently described by the Ostwald power law model when observed at shear rate $50\text{-}1000\text{s}^{-1}$. In the same study, they also observed that viscosity was increased with concentration and decreased with temperature and the impact of temperature on the consistency index was fitted with Arrhenius equation.

1.11 Physical properties of MPC powders

1.11.1 Bulk density and particle size

Bulk density of dairy ingredients is important for both economical as well as functional aspect. Higher bulk density materials occupied less space per unit mass which might be economical in shipping when compared with lower bulk density materials (Sharma et al., 2012). Bulk density decides the container volume, requirement of packaging material and selection of machinery for handling dairy powders (Barbosa-C'novas and Juliano 2005). Bulk density is a measure of the mass of powder which occupies a fixed volume and highly depends on the particle density and internal porosity (Amidon et al., 2017). Porosity indicates the volume fraction of void space inside a material. Tapped bulk density is the highest bulk density that can be achieved without deformation of the particles. (López-Córdoba and Goyanes, 2017). Rupp et al. (2018) mentioned that bulk density of MPC were similar for the samples from different concentration method such as evaporation and NF. A high viscosity of feed in spray-drying modifies powder properties, such as particle size and bulk density and an increase in feed TS usually increases the bulk density of milk powders (Masters, 2002). Crowley et al. (2014a) mentioned that increasing protein content of MPC powders increase the level of interstitial and occluded air results decrease the bulk density. They also mentioned that Particle size of high-protein MPC powder can be reduced by decreasing the viscosity and TS of the MPC in feed prior to spray drying. Most of the milk caseins exist in aggregates or fractions of casein micelles and colloidal calcium phosphate with colloidal particle size in the range of 50-600 nm (Fox and Brodtkorb, 2008). Particle size of skim milk powder was reduced when skim milk was cavitated in HC at 40 Hz prior to

drying (Dahiya, 2016). The size of casein particles was decreased by the application of high-intensity ultrasound (Villamiel and de Jong, 2000, Shanmugam et al., 2012). Skim milk powder and butter milk powder have higher bulk densities compared to MPC and other dairy powders (Silva and O'Mahony, 2017).

1.11.2 Flowability

It is important to understand the behavior of milk powders to store in silos and bags prior to processing (Fitzpatrick et al., 2017). Increased moisture level in the storage period showed negative impact on the flowability of milk powder due to increased liquid bridging and capillary interactions between the particles. Water sorption increased when the lactose is in amorphous state which also reduce the flowability (Fitzpatrick and Processing, 2007). Flowability also reduces due to the migration of fat towards the surface of particle during spray drying and storage period and subsequent liquid bridging (Kim et al., 2005b, Nijdam and Langrish, 2006, Gaiani et al., 2009b). Larger particle size decrease the specific surface and thereby reduce the interparticle interactions results improve the flowability (Fitzpatrick et al., 2004), whereas smaller particle increase the surface area of the powder which increases the cohesive forces between the particles which is considered to reduce the flowability (Reisner and von Rothe Eisenhart, 1971). Increasing protein content in the MPC powders shows poor flowability and requires specific design for the mass-flow hoppers. To utilize as a food ingredient, MPC powders are needed to store in silos prior to use, so it is important to know their behavior during storage and subsequent handlings (Crowley et al., 2014a). Flowability of milk powders can be predicted from Carr's index or Hausner ratio which can be calculated from the

values of loose and tapped density of the powders using equation viii and equation ix, respectively.

$$\text{Carr's index (\%)} = \frac{\text{Tapped density} - \text{Loose density}}{\text{Tapped density}} \times 100 \quad [\text{viii}]$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Loose density}} \quad [\text{ix}]$$

Powder which are not easily flowable during transferring from a conical section of a silo or hoppers where the powder particles initially form a funnel which does not collapse until the mobile powder particles flow through it (Chen et al., 2012), but the particles which remain at the internal walls and cannot flow from the hopper forms rat-holing (Fitzpatrick et al., 2004). For a cohesive powder, a stable arch can form at the hopper outlet during mass-flow and this effect of arching creates a no-flow situation (Iqbal and Fitzpatrick, 2006). As described by past researchers, MPC35 and MPC50 were free-flowing, while MPC60 and MPC70 were easy-flowing (Teunou et al., 1999, Fitzpatrick et al., 2004, Fitzpatrick and Processing, 2007, Crowley et al., 2014a). Crowley et al., (2014) also mentioned that MPC80, MPC85 and MPC90 were cohesive and they claimed that the poor flowability of those powders was due to their small particle size and high specific surface area resulted increased particle–particle interactions and indicated that more cohesive interactions occurred between particles in high protein MPC powders.

1.12 Research project insights and proposed objectives

From the landscape of literature review, it can be said that concentration of MPC80 by NF is superior to evaporation in terms of cost as well as functionality of MPC80 powder after drying. However, the concentration polarization and viscosity can be the issue during NF to concentrate high protein MPC80 at its highest efficiency. Increasing

temperature can reduce the viscosity of high solids MPC80 and can be applied during NF which also helps to break the barrier layer formed due to concentration polarization on the surface of NF. In addition, application of HC helps to reduce the viscosity of high solids MPC80 and can be utilized prior to NF to improve the NF efficiency. It is also considered that HC enhances the functionality of MPC80 and lowers the bacterial load. So, application of elevated temperature or HC or both can have great potential to increase the efficiency of NF during MPC80 concentration by reducing viscosity of feed and thereby concentration polarization during NF. Application of UF in PF module can handle the viscous fluids because of the higher transmembrane pressure and crossflow feeding, which can be utilized to concentrate MPC80. The elevation of temperature in PF system can boost the flow of viscous fluids in more efficient way which can be applied to get higher solids MPC80 in the retentate from PF. Therefore, we hypothesize that increasing temperature either in NF or PF system increases the efficiency of the systems to concentrate MPC80. As per our knowledge, application of high temperature or HC or PF system has not been utilized to concentrate MPC80 and impact of these concentration approaches on the functionality of MPC80 powder also can be the part of study. Therefore, the objectives of this research project were:

1. To evaluate the impact of high temperature and HC on the efficiency of NF to concentrate MPC80.
2. To evaluate the functionality of MPC80 processed from NF.
3. To evaluate the impact of high temperature on the efficiency of PF to concentrate MPC80.
4. To evaluate the functionality of MPC processed from PF.

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CHAPTER 2

Effect of Cavitation and Nanofiltration Temperature on the Production and Quality of Milk Protein Concentrate (MPC80)

INTRODUCTION

Milk Protein Concentrate (**MPC**) is a concentrated form of milk protein containing both casein and whey proteins in the similar ratio as found in skim milk. In general, MPC is produced by filtering skim milk using an ultrafiltration (**UF**) membrane with the molecular weight cut-off of 10-20 kDa (Mistry, 2002, Patel et al., 2014). Higher protein contents, >65% dry basis, can be created using water diafiltration (**DF**) during the UF process, which dilutes the solids and reduces the viscosity which in return helps in the removal of lactose and minerals and thereby increasing the relative concentration of protein to total solids (**TS**) (Guiziou, 2012). An MPC containing 80% protein (**MPC80**) relative to TS is produced by 5 to 8 times volume reduction of skim milk by using UF in conjunction with DF to get a retentate of 20- to 22% TS (Marella et al., 2015). The MPC produced can be further concentrated by using nanofiltration (**NF**) before it is spray dried to save the cost of energy (Schuck et al., 2013). At room temperature, NF can increase the TS of MPC only by 5- to 7% (Cao et al., 2015). There are several hurdles to achieve higher solids during NF. Viscosity of retentate increases with increasing TS during NF (Wang et al., 2018), which further restricts the flow of concentrates in the filtration system. When filtration proceeds, fouling by concentration polarization may develop at the membrane surface which reduces filtration efficiency (Bacchin et al., 2006). Past studies regarding the application of technologies such as cavitation, high pressure homogenization and ultrasonication to MPC showed a reduction of viscosity of feed

during or prior to membrane filtration (Gogate and Pandit, 2005, Sandra and Dalglish, 2005, Patist and Bates, 2008).

Hydrodynamic cavitation (**HC**) is getting popular in dairy processing industries considering its implication in large scale operation, small footprint, easy to operate and cost effective (Tao et al., 2016, Asaithambi et al., 2019). When milk enters into the cavities of an HC rotor, an asymmetric collapse occurs due to high shear stress, results microbial destruction (Paleologou et al., 2007). However, the level of destruction depends on the rotor speed and residence time in the cavitating zone (Save et al., 1994), size and shape of the rotor (Gogate & Pandit, 2005). During HC, apparent viscosity of MPC fluid greatly reduced by disrupting the protein aggregates due to local pressure (Carpenter et al., 2017, Sandra et al., 2019). The higher protein in the feed may induce stronger deposit formation in polymeric membrane (Suárez et al., 2009), which may limits the permeate flux, however, the flux can be increased by increasing temperature (Sood and Kosikowski, 1979). Elevation of temperature helps to reduce the viscosity of milk concentrates containing proteins (Morison et al., 2013). According to the Hagen–Poisellue law, increasing the temperature increases the solute diffusivity and the rate of transport of solutes from the membrane surface into the bulk stream and helps in the dispersion of polarized layer. Application of HC has also been investigated in various food applications for the inactivation of microorganism (Milly et al., 2007, Li et al., 2014) and improving the functionality of MPC powder (Li et al., 2018) and MPC fortified dairy products (Meletharayil et al., 2016). Abovementioned studies showed that either HC, which can be employed between UF and NF, or increased temperature helps to reduce the viscosity of MPC which can improve the efficiency of NF. The objective of

this study was to evaluate the application of HC or elevated temperature (50°C) or both to get the highest level of solids of MPC80 using NF and assess the quality of retentate and powder after drying. In this research, our hypothesis was that, when combined, HC and elevated-temperature treatment during NF could improve the filtration efficiency of MPC80 with retaining the major functional qualities.

MATERIALS AND METHODS

Experimental Design

The production and concentration of MPC80 was carried out at the Davis Dairy Plant at South Dakota State University. We had prepared MPC from UF of pasteurized skim milk. Three replicates of MPC having 20% total solids (TS) containing 80% protein based on total solids (MPC80) were concentrated using NF membrane. Hydrodynamic cavitation or heating or their combined action were applied to liquid MPC80 as treatments. Nanofiltration was carried out at two temperatures: room temperature (22°C), and warm temperature (50°C) whereas HC was carried out at room temperature. We had set the upper temperature limit of NF at 50°C because both the increase of temperature and time of heat treatment might increase the denaturation of whey proteins or their aggregation with casein (Novak, 1992, Qian et al., 2017). Four different NF treatments were utilized including: NF at 22°C (**NF22**); NF at 50°C (**NF50**); HC prior to NF at 22°C (**HCNF22**); and HC prior to NF at 50°C (**HCNF50**). Total time of MPC fluids handling including weighing, preheating, filtration and collecting samples was estimated for 4 hours for each treatment. Milk protein concentrate production variables were evaluated during NF. The retentates were dried in spray dryer to get the MPC80 powder. Retentate and powder samples of MPC80 were refrigerated to perform laboratory analysis.

Ultrafiltration

Pasteurized skim milk (pH range 6.5-6.6) was UF using a commercial polyethersulfone membrane (Parker Hannifin Corp., USA; model SD) with a molecular weight cut-off of 10kDa in a spiral wound design to manufacture the MPC80. The UF process was carried out between 5- to 10°C with an inlet pressure of 385 kPa through four loops. When the protein in the retentate achieved about 65% of TS, DF was started and continued until the final TS reached 20% having 80% protein based on the TS. Approximate composition in the retentate was measured instantly by using an Fourier-transform infrared spectroscopy (Model DairySpec FT, Bentley Instruments Inc., Ireland) to assure the protein content- $\geq 80\%$ based on the TS.

Hydrodynamic Cavitation

For the HCNF22 and HCNF50 treatments, the untreated MPC80 was passed once through an APV Cavitor (SPX Flow Technology, Denmark; model CAV8000) set at 50 Hz and a product flow rate of 100 Lh⁻¹. The cavitor had specially designed rotors with indentations that influenced the flowing track of MPC inside the cavitor. There was total 66 indentations made equidistant from each other on the 203 mm rotor. The gap between the rotor and stator was 3 mm. The feed was passed to the cavitor at a pressure of 13.8- to 17.2 kPa. The average (n=3) inlet and outlet temperatures of MPC80 during cavitation was 7- and 34°C, respectively. For the HCNF22 treatment, the outlet of cavitor was mounted with a heat exchanger system to keep the cavitated product at 22°C.

Nanofiltration

Three replicates of MPC80 were concentrated using NF membrane (Parker Hannifin Corp., USA; model ATF 7938) with a molecular weight cut-off 200 Da in spiral wound design until the permeate flux was $<0.1 \text{ Lm}^{-2}\text{h}^{-1}$. The feed material was preheated indirectly in a water bath to 22°C for NF22 and HCNF22 treatments and 50°C for NF50 and HCNF50 treatments and then NF at that temperature with a 2800 kPa base pressure and 69 kPa boost pressure through a single loop having a diameter 0.2 m, length 0.96 m, area 22.5 m^2 with the spacer 1.65 mm. The total volume of permeate was recorded and collected proportionately to make a permeate composite at the end of filtration. Some portion of final retentate was collected for spray drying. Retentate samples were collected in sterile bottles, sealed immediately and stored at 4°C for 72 h to use for microbial and physicochemical analysis. Some portion of the final retentates and permeate composite samples were stored at -20°C for future use.

Spray Drying

All the NF retentates were dried in a pilot-scale spray dryer (Niro dryer Model 1, Niro Inc., Columbia) having an external mix air atomizing nozzle (Spraying Systems Co., model SUE18A) system. The retentates from NF22 and HCNF22 treatments were preheated indirectly to 50°C in a water bath before spray drying whereas retentates from NF50 and HCNF50 treatments were fed directly to the dryer. To reduce the viscosity and maintain the uniform flow of MPC retentates, an inline heat exchanger was set at 50°C in the dryer feed pipeline system prior to reaching the atomization spray nozzle as described by Oldfield et al., (2000). Retentates were dried to get 2 Kg of powder. The inlet and outlet temperatures used for the drying were set at 175°C and 85°C respectively with the

air flow and pressure $0.00094 \text{ m}^3\text{s}^{-1}$ and 482 kPa and feed flow and pressure 7.5 Lh^{-1} and 240 kPa respectively. Milk protein concentrate powders collected from both cyclone (lighter particles) and main chamber (heavier particles) channels were mixed uniformly and stored at room temperature in airtight containers and used for further analysis. Powder samples for microbial analysis were collected in sterile pouches, sealed immediately and stored at 4°C . Some portion of the powder samples were stored at 4°C in airtight containers for future use.

Production Variables during the concentration of MPC

Total solids of final retentates and permeate composites from NF treatments were measured immediately using Microwave moisture/Solids analyzer (CEM Corporation, North Carolina, USA) with the precision level of 0.01%. Permeate flux was recorded in every 10 minutes until it decreased to $<0.1 \text{ Lm}^{-2}\text{h}^{-1}$. Viscosity of both feed material and final retentate was measured immediately after the collection of samples by using a rotational rheometer (MCR 92, Anton Paar Ltd., UK) fitted with bob-and-cup configuration. The viscosity was observed at 22°C and 50°C at constant shear rate of 100 s^{-1} . Volumetric concentration ratio (VCR) was calculated as the ratio of total feed to total retentate.

Compositional Analysis

Samples of feed, retentate, and permeate composite from MPC manufacture were collected and immediately cooled at 4°C . Total nitrogen, non-casein nitrogen (NCN) and non-protein nitrogen (NPN) contents were determined from Kjeldahl method as mentioned in AOAC (2000a). Total protein was calculated by multiplying by a factor 6.38 with the total nitrogen and expressed as percentage of total dry solids. Lactose

content was measured using an HPLC-based method, as described by Amamcharla and Metzger (2011). Crude fat content was measured by Modified Mojonnier method and total ash by gravimetric method as mentioned in AOAC (2000b). Mineral analysis of the samples was done using inductively coupled plasma-optical emission spectroscopy as mentioned in AOAC (2005).

Protein Fractions

Samples of skim milk, feed material, and retentate and permeate composite from NF were analyzed for protein fractions using capillary electrophoresis system (Beckman P/ACE MDQ, Beckman Coulter, Fullerton, CA, USA) equipped with a UV detector set at 214 nm. Sample preparation was carried out according to the method described by Salunke et al., 2011. Samples were diluted with HPLC grade water to get $\leq 0.1 \text{ mgmL}^{-1}$. Separation was obtained via a $50 \mu\text{m}$ bare fused silica capillary with the length of 30.2 cm. For the estimation of protein molecular weights in the sample, the SDS-MW size standard (recombinant proteins 10 to 225 kDa supplied with the ProteomeLab SDS-MW Analysis Kit) was used to estimate the molecular weight of the proteins in each sample. β -mercaptoethanol ($5 \mu\text{L}$) was added to each microfuge vial containing diluted SDS-MW size standard ($10 \mu\text{L}$ in $85 \mu\text{L}$ of sample buffer). Prepared vials were heated in a water bath for 10 min at 90°C . A separation at constant voltage of 15 KV, 25°C temperature and 20 bar pressure was performed with reverse polarity in SDS-MW gel buffer for 30 min. A capillary preconditioning was done in six-run cycle. Sample was injected electrokinetically for 20 s at 5 KV. To operate the program, a 32 KaratTM Software (Beckman Coulter, Inc.) was used. After the completion of run, protein peaks and area covered were identified from the electropherogram and percentage of protein

fractions were calculated. Area of the peaks between solvent's peak and the peak of α -LA were totalized and calculated as low molecular weight peptides (LMWP) and area of peaks after the peak of κ -CN in the electropherogram were totalized and calculated as high molecular weight peptides (HMWP).

Physical Properties of MPC Powders

Relative dissolution index (**RDI**) of the MPC powders stored at 4°C for 6 months were evaluated using focused beam reflectance measurement (**FBRM**) following the method described by Hauser and Amamcharla (2016). Powder sample of MPC was dissolved in deionized water maintained at 25°C to make 5% (wt/wt) protein concentration in the solution. A glass beaker (250 ml) equipped with an overhead stirrer having 4-blade impeller (Caframo, Georgian Bluffs, Ontario, Canada) was used to dissolve the powder particles at 400 rpm. For acquiring and monitoring the FBRM data, an iC FBRM software (version 4.3.391, Mettler-Toledo AutoChem Inc., Columbia, MD) was used. The software program helped to track the number of fine particles having the chord length <10 μ m. The dissolution characteristics of MPC powders were monitored using changes in particle counts for 30 min. When MPC powder started dissolution, the counts of fine particles were increased with time. The fine particle counts were then mapped against powder dissolution time. Further, the area under the fine particle count curve was calculated by using the trapezoidal rule to describe the powder dissolution characteristics. RDI (%) of MPC powder was calculated by using equation [1].

$$\text{RDI (\%)} = \frac{\text{Area under the curve for the NF treatment}}{\text{Area under the curve for the UF-milk}} \times 100 \quad [1]$$

Bulk density of MPC powder was measured for both loose and tapped condition (IDF Standard 134A:1995). For the loose density, MPC powder was poured in a dry pre-weighed 100 mL calibrated glass cylinder up to the mark of 100 mL without any shaking and then weighed. After weighing, the same cylinder was tapped for 100 times using Bulk Density Apparatus (UNILAB-009, India) and then volume after tapping was measured. Loose and Tapped bulk densities were calculated by using equation [2] and [3] respectively.

$$\text{Loose Bulk Density} = \frac{\text{Weight of powder (Kg)}}{\text{Volume of powder before tapping (m}^3\text{)}} \quad [2]$$

$$\text{Tapped Bulk Density} = \frac{\text{Weight of powder (Kg)}}{\text{Volume of powder after tapping (m}^3\text{)}} \quad [3]$$

Morphological characteristics of MPC powder such as diameter (circle equivalent diameter), circularity (how close the shape of the particle to a perfect circle), elongation (ratio of width to length), solidity (ratio of particle area to convex hull area) and convexity were calculated from the 2-D images by using Malvern Morphologi G3ID (Malvern Instruments, Worcestershire, UK).

Microbial Examination

Viable microorganisms existed in the NF-retentates and MPC80 powders was enumerated as SPC by standard agar method as described by Wehr and Frank (2004). Retentate samples were measured in gram instead of milliliter, because the gel was solidified after refrigeration, and diluted 100 times (wt/v) with sterilized phosphate buffer saline PBS. For the reconstitution of MPC80 powder, 11 g powder sample was mixed with 99 mL PBS in stomacher bag and thoroughly agitated using stomacher machine and further diluted 100 times (v/v) with PBS by serial dilution. Diluted samples were pipetted

out (100 μ L) and mixed aseptically with sterilized standard agar (10 to 12g) media at 45°C by pour plate technique in petri-plates. Plates were incubated at 37°C for 24 h and colonies were counted as Colony-forming units (CFU) using colony counter and the count was multiplied by the reciprocal of the dilution and expressed as $\log_{10}\text{CFUg}^{-1}$.

Aerobic mesophilic spores (AMS) in MPC powder was enumerated by using Tryptic Soy Agar (TSA) media as described by Kent et al., (2016). Reconstituted sample (11g powder in 99mL PBS) was heated to 80°C for 12 min, cooled to room temperature and diluted 100 times (v/v) with PBS by serial dilution. Diluted samples were pipetted out (100 μ L) and mixed aseptically with sterilized TSA media (10 to 12g) media at 45°C by streak plate technique in petri-plates. Plates were incubated at 37°C for 48 h and colonies were counted as CFU using colony counter and the count was multiplied by the reciprocal of the dilution and expressed as $\log_{10}\text{CFUg}^{-1}$.

Statistical Analysis

Statistical analysis of replicates data was performed using Agricolae: Statistical Packages for Agricultural Research in R programming language (version 3.5.2). Tukey's Honest Significant Difference (HSD) test was used to determine differences between treatment means and the level of significance was decided at $P < 0.05$.

RESULTS AND DISCUSSION

Production Variables

Average permeate-flux, average volumetric concentration ratio, viscosity of feed and final retentates and TS of final retentates were evaluated during NF of MPC (Table 1). High temperature had a significant ($P < 0.05$) effect on the average flux and final TS of

retentates. The average permeate flux at higher temperature (50°C) NF was increased by more than 98% when compared to the low temperature NF treatments. The final TS for the high temperature NF retentates with and without cavitation was increased by 17 to 26% when compared to the NF22 having the final TS of 25.08%. When solids built up in the retentate, the flux was decreased continuously and become less than $0.1 \text{ Lm}^{-2}\text{h}^{-1}$ which might be due to increasing fouling on membrane. Small particles in mixtures contribute to flux decline by blocking surfaces and pores of membrane (Tarleton et al., 1994). As described by Lipnizk et al., (2005) and Meyer et al., (2015), a huge pressure drops occurs when filtration progress during the processing of high protein feed in spiral wound membrane which is mainly due to the adsorption of proteins on the membrane resulting very low permeate flux, which was agreed to our results.

Higher flux for the high temperature treatments showed that there was a significant reduction in processing time. High temperature lowered the viscosity which might be facilitated to improve flux. We did not notice any past research for MPC concentration at high temperature NF. As described by Sood and Kosikowski (1979), high temperature increased diffusivity, and helped to disperse the polarized layer which improved the UF efficiency. Unlike high temperature, HC did not have a significant ($P > 0.05$) impact on average flux but did significantly increase ($P < 0.05$) the final TS (Table 1). The apparent viscosity after cavitation was reduced greatly (data not shown) which may possibly helped in easy flowing of feed on membrane surface resulting elongation of NF and built more solids in the final retentates. Impact of HC was not a significant ($P > 0.05$) on average VCR at 22°C but that was significant ($P < 0.05$) at high temperature. Application of HC here reduced the viscosity of feed apparently prior to NF. The amount of permeate

was increased when feed was heated to 50°C and maintained the same temperature during NF and contributed to increase VCR. The higher permeate amount from high temperature NF might be due to the less concentration polarization on membrane surface and better diffusivity of the feed during NF.

With increasing TS, there was a significant ($P < 0.05$) increase in viscosity; 11, 61, 237, 76 and 284 cP, for the feed, NF22, NF50, HCNF22 and HCNF50 retentates when measured at 50°C. Several past research of ultrafiltration of skim milk showed that the viscosity of skim milk increases exponentially with increasing concentration (Meyer et al., 2015, Meena et al., 2016). The increased viscosity can increase shear, pressure drop, and deposit layer formation which ultimately reduces the flux (Akoum et al., 2005, Lipnizki et al., 2005). When the total milk protein increases during filtration which come with an increase in viscosity, at this point the work of NF membranes at lower temperatures would be limited (Solanki and Rizvi, 2001). To overcome this limitation, we employed NF of MPC at higher temperature (50°C) which significantly ($P < 0.05$) reduced the viscosity of feed and improved the VCR and level of final TS in the retentate.

Composition of retentates

Composition of feed and final retentate were evaluated based on percentage of dry solids (Table 2). Ideally, the NF membrane does not allow protein, fat, and lactose in the permeate. There was a slight reduction of protein in the retentates from high temperature NF, however the reduction was not significant ($P > 0.05$). Both HC and high temperature did not have a significant ($P > 0.05$) impact on the lactose and crude fat contents. Except fat content, the composition assessed in this study was comparable to ADPI standards; protein 79.5%, fat 2.5%, lactose 9.0%, ash 8.0% on dry weight basis (ADPI, 2020). A

slightly higher fat content was recorded in our MPC80 samples which might be due to the improper fat skimming of milk prior to UF.

The protein content in the final retentates was in the range of 78.10 to 79.69% on dry weight basis which was like feed and the values were statistically similar ($P > 0.05$). Both high temperature and HC impacted significantly ($P < 0.05$) on the total ash content of retentates. As expected, when NF proceeds, less retention of monovalent ions (IDF, 2019) compared to divalent ions (Suarez et al., 2006) which helps to maintain the level of protein in the retentate. Results of total ash showed that both HC and high temperature NF impacted to drain the minerals in the permeate. However, total ash in the retentate from NF22 was significantly ($P < 0.05$) higher compared to other treatments which might be due to the lower passage of monovalent ions in the permeate. Similarly, both HC and high temperature did not have a significant ($P > 0.05$) impact on NCN in the retentates of NF. Since there is not a significant treatment effect on NCN levels, it is expected that the similarities in total protein are recognized as NCN which is the combination of whey protein and NPN. But in our studies, NPN was increased significantly ($P > 0.05$) on high temperature NF retentates. The NPN of bovine milk is composed of urea (~50%), free amino acids, creatine, creatinine, uric acid, peptides, orotic acid, and ammonia (Wolfschoon-Pombo, and Klostermeyer. 1981). Our results showed that the level of NPN was increased (0.48 to 0.66%) in the high temperature NF which might be due to the increased proteolytic activity at higher temperature as described by Bu et. al., (2013). One of the research related to sheep and goat cheese whey NF showed the apparent rejection coefficients of TS, total nitrogen (and consequently crude protein), NPN, and lactose were almost constant (Macedo et al., 2018). They also mentioned that rejection of

NPN (mainly amino acids) decreased during the process of NF by 8%, which was agreed to our results when compared with low temperature NF.

Composition of Permeates

Total solids of permeate composites from four different NF treatments were analyzed. Further, the components of total solids in the permeate such as total protein including NCN and NPN, lactose and total ash contents were analyzed based on the percentage of TS (Table 3). Results showed that high temperature had a significant ($P < 0.05$) impact on the total solids of permeates. Nanofiltration membranes are specifically designed to allow water, ions, and minerals and recover the larger organic molecules as a retentate. However, the recovery rate of larger organic molecules also depends on the operating temperature, pressure, and VCR (Cassano et al., 2018). Some of the past research showed that protein retention was nearly 100% for all the operating conditions tested in NF of milk or whey, while lactose retention decreased from 99.8% to 97.5% as the concentration boosted (Meyer et al., 2015, Suarez et al., 2006). The NF membranes applicable to milk and milk products filtration generally exhibit a high permeability for monovalent ions (between 40 to 90%), and a low or very low permeability for multivalent ions (between 5 to 20%) and organic compounds (proteins, lactose, urea) (Kelly and Kelly, 1995). In the current research, small amount of total nitrogen (maximum 0.05% on as is basis) was drained in the permeate of NF treatments. Permeates obtained from the high temperature NF had a slightly lower total nitrogen as well as NPN when compared with low temperature NF. Similarly, high temperature NF significantly ($P < 0.05$) increased the removal of lactose in the permeates, and similar results were observed by Meyer et al., (2017). Organic nitrogen compound such as urea

can be passed with permeate through the RO or NF membrane by convective transport method (Lee and Lueptow, 2001), which might be contributed on the total nitrogen as well as NPN.

The lower total nitrogen in the permeates of high temperature NF might be due to the dilution effect because the amount of permeate was higher at high temperature in which the draining of lactose and its derivatives was increased with increasing VCR, and similar information has been described by Atr'a et al., (2005). The charge effect would be applied in the transport phenomena in NF when charged (ions) and uncharged (lactose, sugar etc.) solutes are present in the feed (Bowen and Welfoot, 2002). Another research of NF conducted for separating glucose and salts also yielded similar results; glucose retention decreased when the level salt concentration increased (Bargeman et al., 2005). From this study, we did not see any impacts ($P > 0.05$) of HC and high temperature on the NCN in permeates of all treatments. There was considerable loss (20%) of NPN in the permeate was also recorded in a research (Romain et al., 2000). Similar other research findings such as; the efficiency of NF process was affected by the passage of nitrogen compounds to permeate (Minhalma et al., 2007), the loss of nitrogen compounds to permeate is reliant on the characteristics of the membrane (Brans et al., 2004) and can be influenced by pretreatments of feed and the operating conditions of the NF process (Alkhatim et al., 1998), also supported to our NPN results.

Bovine milk contains several minerals (Haug et al., 2007), however, during MPC production, most of the minerals are washed from the milk during UF/DF process. Our research showed that the level of total ash content was 20 to 39% based on permeate TS. Total ash content in the permeate was decreased significantly ($P < 0.05$) when NF was

conducted at high temperature, but HC impacted significantly ($P < 0.05$) to increase the level of ash in the permeate. One of the research showed that the total ash content in whey reduced by 3 to 4 times when concentration of whey was done by using nanofiltration (Kelly and Kelly, 1995). There is limited research on the impact of HC regarding mineral retention during NF. Some of the studies related to whey and dairy effluents processing by using NF showed that more minerals could be removed by lowering the pH of feed material (Pan et al., 2011, Macedo et al., 2018).

Protein Fractions of Retentates

Protein fractions based on the total protein of feed and NF retentates were compared with the skim milk (Table 4). Low molecular weight peptides (LMWP) (MWCO < 14 kDa) fraction (3.23 to 3.50%) in the NF retentates was reduced significantly ($P < 0.05$) when compared to skim milk and feed. Results of LMWP indicated that there was increasing microbial growth at our NF processing temperatures resulting proteolysis of caseins. The proteolytic activity on milk protein formed low molecular peptides and amino acids (Mutilangi et al., 1995) which can be permeated through the NF membrane.

Whey proteins mainly comprised by β -LG (~50%) and α -La (~20%). Current research showed that both HC and high temperature NF did not have an impact on α -LA content, but β -LG was reduced significantly ($P < 0.05$) in the NF50 and HCNF50 treatments. The level of γ -CN was increased significantly ($P < 0.05$) at high temperature NF. Protein fraction such as γ -CN can be formed from the proteolytic degradation of β -CN (Eskin and Goff, 2013) and the degradation can be induced by higher processing and or storage temperature (Ismail and Nielsen, 2010).

Level of major casein proteins; α_{s1} - and β -CN were increased significantly ($P < 0.05$) in the retentates of NF compared to feed, but differences was not a significant ($P > 0.05$) among the NF treatments. Similarly, there was not a significant difference ($P < 0.05$) in α_{s2} -CN among the feed and NF-treatments whereas the retention of K-CN was reduced and high molecular weight peptides (HMWP) was increased significantly ($P < 0.05$) in HC treated samples. The majority of denatured β -LG in high pressure treated milk was associated with casein micelles and may have significant impact on the properties of products made from such type of milk (Anema et al., 2003, Huppertz et al., 2004). Casein molecules undergo chemical modifications mainly with sugars, due to the free amino groups lysyl residues (Camille and Gaucheron, 2015). In the current research, HC as well as high temperature might be favored to increase the level of HMWP. It is considered that HMWP in bovine milk mainly derived from the enzymatic hydrolysis of bovine serum albumin (Huang et. al., 2004). Results of protein fractions in MPC powders such as β -LG (~9%), α_{s2} (~7%), and β -CN (32-34%) from past research (Sikand et al., 2011) were comparable to our results. Since we had tested the protein fractions in the MPC retentates before drying, the level of α_{s1} (~38%) and K-CN (9-10%) were relatively higher which might be the effect of drying (Fang et al., 2012). We did the protein fraction assessment in the permeate composite samples from all the NF treatments, but only LMWP fractions were detected (data not shown). The LMWP fractions can be developed by the action of microbial proteases on whey proteins (Contesini et al., 2018) and the degradation accelerated at high temperature (Vázquez-Lara et al., 2003).

Microbial Examination

Standard plate counts (SPC) of feed and retentates was assessed to see the level of bacterial load after the completion of NF. Similarly, SPC and aerobic mesophilic spores (AMS) of MPC powders after spray drying were examined to confirm the level of acceptability compared with the international standards. Both SPC and AMS were expressed in terms of \log_{10} value of colony forming units per gram sample ($\text{Log}_{10}\text{CFU/g}$) (Figure 1). The SPC in the retentates prior to drying were 4.63, 4.93, 4.88 and 5.42 $\text{Log}_{10}\text{CFU/g}$ for the NF22, NF50, HCNF22, and HCNF50 treatments, respectively. Retentates from high temperature NF treatments had significantly ($P < 0.05$) higher SPC compare to low temperature NF treatments (Figure 1). Single pass HC did not make any impact on the reduction of SPC in the retentates. As in the previous studies mentioned, the impact of HC on the microbial destruction increased with increasing number of passes through the cavitating zone or higher discharge pressure or both (Chaudhary, 2019; Save et al., 1994). We had applied the pressure 13.79 to 17.24 kPa during cavitation which was too low to destruct the microorganisms. For the microbial destruction, it requires the pressure >300 MPa (O'Reilly et al., 2000). Studies also showed that, the destruction rate also depends on the initial load of microbes. As our feed was stored at 5°C until cavitation, the load of microorganisms was likely to be low ($3.47 \text{ Log}_{10}\text{CFU/g}$) and when the cavitation proceeded, the environment for their multiplication became more favorable. Similar effects were observed when cell suspension was cavitated at different concentration and pressure levels in the yeast (Save et al., 1994) and in zooplankton of seawater (Paleologou et al., 2007). The load of SPC was higher in the MPC powders from NF treatments were statistically similar ($P > 0.05$) among the NF

treatments ranging 2.28 to 2.67 Log₁₀CFU/g but were higher compared to feed (0.86 Log₁₀CFU/g), however, it was under the acceptable limit as described by USDA dairy standards (USDA, 2001). Our result also agreed to the results of a research conducted for the microbial examination of thermophilic bacteria showing an average bacterial count 2.57 Log₁₀CFU/g in the non-fat dry milk powder samples collected from the Midwest region of United States (Buehner et al., 2015).

In milk, some of the bacteria including *Bacillus pumilus*, and *B. licheniformis* usually grow at temperature range 30 to 40°C and sometimes as high as 46°C while *Anoxybacillus* spp. and *Geobacillus* spp. are considered obligate thermophiles and usually grow at temperature 50 to 62°C and 55 to 65°C respectively (Rueckert et al., 2006, McHugh et al., 2017). These microorganisms have the capability to form and withstand as spores in dry milk powder and its products and can grow in favorable condition and functioned as a live cell (Doyle et al., 1997). Research verified that mesophilic spores are the most common sporeformer found in bulk tank raw milk (Miller et al., 2015). In the current research, AMS count in the MPC powder from all the treatments were statistically similar ($P > 0.05$) and found in the range of 2.15 to 2.88 Log₁₀CFU/g sample. Similar results were observed in skim milk powder samples (Ali et al., 2013) and whey protein concentrate, non-fat dry milk and skim milk powder samples (Kent et al., 2016). In a study, pasteurization processes between the temperatures of 72 and 76°C caused more spores to be activated than pasteurization treatments at other temperatures (Hammer et al., 1995, Hanson et al., 2005). The range of spore counts in MPC powder from all NF treatments was under the acceptable limit as described by US Dairy Export Council ($< 3 \text{ Log}_{10} \text{CFU/g}$).

Mineral Analysis

Minerals perform a key role in the structure and stability of milk protein, mainly casein micelles. Macro minerals such as Ca, P, Na, K, Mg and S which are available in the MPC powder of feed and final retentates were assessed (Table 5). Macro minerals such as Ca, Na, K and Mg are an essential group of milk nutrients with the recommended daily intake > 100mg per day for optimal function (WHO, 1996), were available in significant amount in the MPC. Salt/free minerals can be passed with permeate from both UF/DF and NF process. Some of the minerals in ionic form involved in the formation of polarized layer on the surface of membrane and can be retained in the retentates. Other minerals which involved in the synthesis of organic compounds mainly protein contribute to the mineral composition of MPC.

In our studies, both HC and high temperature did not impact significantly ($P > 0.05$) on the individual minerals such as Ca, P, Na, K, Mg retention in all kind of NF treatments. MPC is the rich source of casein, a native micellar form, which holds substantial amounts of calcium and phosphorus (O’Kennedy, 2009) and remain relatively unchanged during UF/DF and NF whereas the serum phase changes considerably results increased in pH and decrease in ionic strength (Huppertz et al., 2018). According to previous research, MPC powder contained about 2% calcium when the level of protein >65% (Rehman et al., 2003), which is agreed to our results. When we compared to another similar kind of past study having the average contents of Ca~1500mg, Mg~80mg, K~350mg, Na~100mg and P~1100mg in the MPC80 powder samples (Sikand et al., 2011), the level of these minerals from our findings were relatively higher. Sulphur content was increased significantly ($P < 0.05$) in the NF retentates compared to feed and

among the NF retentates, the concentration was slightly higher in the retentates from high temperature NF. When the level of total protein decreased the amount of S content can be increased. The condition might be due to the relative abundance rather than the evolution of new compounds (Drake et al., 2014) and the statement is supportive to our results. The relative protein content in the retentates from high temperature NF treatments was slightly lower (Table 2) in which the corresponding S content was found higher compared to room temperature NF treatments.

Physical Properties of MPC Powders

Results of RDI suggested that both HC and high temperature influenced in the dissolution characteristics of MPC powder (Table 6). Powder sample from HCNF22 treatments showed relatively higher RDI (92.33%) whereas the treatment NF50 showed lowest RDI (70.57%) as compared with powder from feed sample which was assumed as 100%. One of the studies of MPC processed in HC revealed that there was a significant reduction on the particle size of casein when MPC was cavitated at rotor speed 40 Hz (Dahiya, 2016). Though, in our study we had operated the HC at 50 Hz, the results agreed to the previous studies and similar other literatures (Sutariya et al., 2018). The MPC powders having lowest particle size (diameter, 8.93 μ m) from HCNF22 (Table 6) among the treatments demonstrated the highest RDI. Dahiya (2016) also mentioned that MPC samples subjected to high temperature processing with or without HC did not change or increased the particle size of casein, which was also agreed to our results. The largest particle size (diameter, 9.51 μ m) of MPC powder from NF50 treatment had the lowest RDI which indicated poor dissolution characteristics. Casein micelle size can be increased at high temperature processing due to heat denaturation of whey proteins and

then forming a complex between denatured whey proteins and κ -CN (Anema and Li, 2003). This kind of reactions are dependent on environmental conditions and temperature, and the type and concentration of heat induced complexes (Corredig et al., 2019). Though the temperature was only moderate for the treatments NF50 and HCNF50, the exposure time and pressure might induced the formation of whey-casein interaction to increase their size to some extent, however the changes was not significant ($P > 0.05$).

Bulk density of MPC powder was measured for both loose and tapped condition. Our study showed that the combination of HC and high temperature did not have a significant ($P > 0.05$) impact on loose density of MPC powders but tapped density was significantly ($P < 0.05$) higher for the HC treated samples (Table 6). As mentioned by the Li et al., (2018), HC impacted significantly on both loose and tapped densities of MPC powders when it was applied after UF or NF. A similar trend was seen in our results mainly on tapped density though we applied the HC before NF. The loose density was highest for the MPC powder from HCNF50 treatments and this result confirmed that the volume of occluded air in the HC treated samples might be less compared with the samples without HC treatments. The lower particle size of HC treated MPC powder can be a factor of higher bulk density. In addition, internal porosity of powder particles and arrangement of particles also matters on the density of dairy powders (Sharma et al., 2012). Loose and tapped densities of food powders are important during storage, packaging, shipping, handling, and further processing (Barbosa-C'anvas and Juliano 2005).

The morphological properties of MPC powders from different treatments were investigated by using light microscopy with image processing for circularity, aspect ratio, elongation, solidity, and convexity and the results has been summarized in Table 6.

Convexity is a measure of the surface roughness of a particle. A smooth particle has a convexity ratio equivalent to one while an irregularly shaped particle has a convexity closer to zero. Similarly, fully circle has an elongation value zero but, shapes with large aspect ratios have an elongation ratio closer to one (Li et al., 2016, Babu and Amamcharla, 2018). Both HC and high temperature did not have a significant ($P > 0.05$) impact on the physical properties of MPC powder particle such as diameter, circularity, aspect ratio, elongation, solidity, and convexity. Li et al., (2016) found that higher convexity and solidity of MPC powder showed better dissolution properties, however, the trend may not be followed when the level of protein in MPC powder increased (Babu et al., 2018). In the current study, higher solidity was recorded for the HC treated powders, though the difference was not significant ($P > 0.05$), and the corresponding RDI results also supported that HC treated samples had good dissolution characteristics.

The changes in the fine particles counts of MPC powder samples which were tracked from the chord length of less than 10 μm was monitored for 30 minutes and the result has shown in Figure 2. The powders from all the treatments showed similar trend; increased fine particle counts in FBRM with time. For the first 5 minutes, samples from all NF treatments showed higher counting compare to feed samples. After 5 minutes, counting rate for the feed sample increased rapidly with 90,000 counts within 30 minutes. Powders from NF22 and HCNF22 had about 75,000 counts whereas powders from NF50 and HCNF50 had less than 70,000 counts. In overall, fine particle counts when exposed to deionized water increased more rapidly for feed sample (processed at $<10^{\circ}\text{C}$). Powders from NF22 and HCNF22 (processed at 22°C) had similar counting rate which was higher than the counts from NF50 and HCNF50 (processed at 50°C) treatments. When MPC

exposed at high temperature for longer time, a crosslinking networks developed around the protein particles due to the interactions between hydrophobic caseins and whey proteins which leads to the poor dissolution characteristics (Anema et al., 2006). When protein content increased, there is high chances of protein particle crosslinking and showed more resistance to dissolve in water (Crowley et al., 2015). In the current studies, the data were comparable to the overall trend as mentioned in the similar previous studies (Crowley et al., 2015, Hauser and Amamcharla, 2016) when compared to the dissolution rate.

CONCLUSIONS

This study investigated the implication of HC and high temperature on the filtration performance of MPC80 in NF system and assessed their impacts on the quality. High temperature impacted greatly to increase the permeate flux as well as the level of TS in the retentate whereas HC contributed only on increasing TS of retentate. With increasing the level of solids in the retentates of high temperature NF, the draining of solids to the permeate also increased by 0.2%. For all the NF treatments, the level of protein retention was $\geq 99.95\%$. The load of SPC in the retentate was increased at high temperature NF within 4 h of total working, however, both SPC and AMS counts in the MPC80 powders were within the acceptable limit as described by US dairy standards. Combination of HC and high temperature contributed to increase the tapped density of MPC80 powder which might reduce the packaging, handling, and storage cost of MPC powder. Unlike high temperature, HC improved the dissolution characteristics of MPC80 powder due to the decrease in average particle size. This study determined that high temperatures or their combined action with HC improve nanofiltration performance whereas HC alone

improves the functional quality of MPC80. Overall, the findings of this study help to reduce the cost of MPC80 drying with maintaining the quality.

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Table 1. MPC Production variables (mean, n=3)

Treatments ¹	Flux ² (L/m ² h)	VCR ³	Viscosity ⁴ at 22°C (cP)	Viscosity ⁴ at 50°C (cP)	Total Solids (%) wt/wt)	Total Protein ⁵ (%)
Feed	-	-	68 ^e	11 ^c	20.31 ^e	79.69 ^a
NF22	1.55 ^b	1.36 ^c	248 ^d	61 ^b	25.08 ^d	79.10 ^a
NF50	3.18 ^a	1.49 ^b	959 ^b	237 ^a	29.49 ^b	78.06 ^a
HCNF22	1.58 ^b	1.34 ^c	541 ^c	76 ^b	26.83 ^c	78.62 ^a
HCNF50	3.13 ^a	1.63 ^a	1302 ^a	284 ^a	31.49 ^a	78.10 ^a

^{a-e}Values with the same superscript within a column are not significantly different (P > 0.05).

¹Feed was an MPC prepared from ultrafiltration of skim milk; NF22 and NF50 were retentates from nanofiltration of feed at 22 and 50°C; HCNF22 and HCNF50 were retentates from the combined processing of hydrodynamic cavitation and nanofiltration of feed at 22 and 50°C.

²Average permeate flux during nanofiltration

³Average volumetric concentration ratio during nanofiltration

⁴Viscosity of feed or final retentates from nanofiltration

⁵Total Protein based on total dry solids of retentates

Table 2. Mean (n=3) compositional analysis of MPC retentates based on total solids

Treatments ¹	Total Protein %	Lactose %	Total Ash %	Crude Fat %	NCN ² %	NPN ² %
Feed	79.69 ^a	7.42 ^b	8.38 ^a	4.04 ^a	2.84 ^a	0.44 ^b
NF22	79.10 ^a	9.58 ^a	8.30 ^a	3.35 ^a	2.87 ^a	0.48 ^b
NF50	78.06 ^a	9.39 ^a	7.65 ^b	3.80 ^a	2.84 ^a	0.66 ^a
HCNF22	78.62 ^a	9.34 ^a	7.55 ^b	3.98 ^a	2.81 ^a	0.49 ^b
HCNF50	78.10 ^a	9.78 ^a	7.66 ^b	4.01 ^a	2.87 ^a	0.66 ^a

^{a-b}Values with the same superscript within a column are not significantly different (P > 0.05).

¹Feed was an MPC prepared from ultrafiltration of skim milk; NF22 and NF50 were retentates from nanofiltration of feed at 22 and 50°C; HCNF22 and HCNF50 were retentates from the combined processing of hydrodynamic cavitation and nanofiltration of feed at 22 and 50°C.

²NCN=Non-Casein Nitrogen, NPN= Non-Protein Nitrogen.

Table 3. Mean (n=3) compositional analysis of MPC permeates based on total solids

Treatments ¹	Total Solids	Total Protein	NCN ²	NPN ²	Lactose	Total Ash
NF22	0.29 ^b	15.13 ^{ab}	2.25 ^a	1.92 ^a	45.29 ^c	38.72 ^a
NF50	0.47 ^a	13.69 ^{bc}	2.18 ^a	1.34 ^b	65.89 ^a	19.60 ^c
HCNF22	0.32 ^b	17.34 ^a	2.41 ^a	1.99 ^a	52.23 ^b	29.25 ^b
HCNF50	0.50 ^a	11.86 ^c	2.69 ^a	1.55 ^b	63.66 ^a	24.05 ^{bc}

^{a-c}Values with the same superscript within a column are not significantly different (P > 0.05).

¹NF22 and NF50 were the permeates from nanofiltration of feed (MPC prepared from ultrafiltration of skim milk) at 22 and 50°C; HCNF22 and HCNF50 were the permeates from the combined processing of hydrodynamic cavitation and nanofiltration of feed at 22 and 50°C.

²NCN=Non-Casein Nitrogen, NPN= Non-Protein Nitrogen.

Table 4. Protein fractions (% , mean, n=3) of skim-milk¹ and retentates²

Proteins ³	Skim-milk	Feed	NF22	NF50	HCNF22	HCNF50
LMWP	4.25 ^{ab}	4.76 ^a	3.23 ^c	3.42 ^{bc}	3.50 ^{bc}	3.34 ^{bc}
α -LA	4.21 ^a	3.44 ^{abc}	3.30 ^{bc}	2.60 ^c	3.05 ^{bc}	3.51 ^{ab}
β -LG	9.61 ^{ab}	9.81 ^a	9.01 ^{abc}	7.66 ^c	8.58 ^{abc}	8.29 ^{bc}
γ -CN	0.86 ^c	0.91 ^c	0.94 ^{bc}	1.33 ^{ab}	0.75 ^c	1.43 ^a
β -CN	35.22 ^{ab}	34.19 ^b	35.99 ^a	35.39 ^{ab}	35.38 ^{ab}	34.57 ^{ab}
α_{s1} -CN	34.57 ^{ab}	33.75 ^b	34.28 ^{ab}	35.52 ^a	35.16 ^{ab}	34.56 ^{ab}
α_{s2} -CN	5.40 ^b	7.93 ^a	7.72 ^a	8.25 ^a	8.09 ^a	9.20 ^a
κ -CN	4.35 ^a	3.53 ^{bc}	4.11 ^{ab}	4.39 ^a	3.93 ^{abc}	3.30 ^c
HMWP	1.54 ^{ab}	1.37 ^b	1.41 ^b	1.44 ^{ab}	1.56 ^{ab}	1.81 ^a

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹Skim-milk was the source material for feed and retentates of respective treatments.

²Retentates= Feed was retentate from ultrafiltration of skim milk; NF22 and NF50 were the retentates from the nanofiltration of feed at 22 and 50°C; HCNF22 and HCNF50 were the retentates from the combined processing of hydrodynamic cavitation and nanofiltration of feed at 22 and 50°C.

³Proteins=protein fractions: LMWP=low molecular weight peptides; α -La=alpha lactalbumin; β -LG= beta Lactoglobulin; γ -CN=gamma casein; β -CN=beta casein; α_{s1} -CN=alpha casein-1; α_{s2} -CN =alpha casin-2; κ -CN=kappa casein; HMWP=high molecular weight peptides.

Table 5. Mineral analysis (mg/100g, mean, n=3) of MPC powder¹

Treatments ²	Ca	P	Na	K	Mg	S
Feed	2140 ^a	1503 ^a	205 ^a	508 ^a	122 ^a	17 ^b
NF22	2012 ^a	1159 ^a	221 ^a	526 ^a	117 ^a	30 ^{ab}
NF50	2228 ^a	1287 ^a	243 ^a	559 ^a	132 ^a	34 ^a
HCNF22	2231 ^a	1434 ^a	244 ^a	576 ^a	131 ^a	27 ^{ab}
HCNF50	2049 ^a	1173 ^a	237 ^a	477 ^a	121 ^a	31 ^a

^{a-b}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹MPC powder was prepared from spray drying of MPC

²Feed is an MPC powder of retentate from the ultrafiltration of skim milk; NF22 and NF50 are MPC powders of retentates from the nanofiltration of feed at 22 and 50°C; HCNF22 and HCNF50 are MPC powders of retentates from the combined processing of hydrodynamic cavitation and nanofiltration of feed at 22 and 50°C.

Table 6. Physical properties (mean, n=3) of MPC powder¹

Properties	Feed ²	NF22 ²	NF50 ²	HCNF22 ²	HCNF50 ²
RDI ³	100 ^a	86.04 ^{ab}	70.57 ^c	92.33 ^a	73.99 ^{bc}
Loose Density (Kgm ⁻³)	266 ^a	273 ^a	278 ^a	272 ^a	286 ^a
Tapped Density (Kgm ⁻³)	328 ^c	344 ^{bc}	348 ^b	356 ^{ab}	366 ^a
Particle Diameter (µm)	9.12 ^a	9.30 ^a	9.51 ^a	8.93 ^a	9.49 ^a
Circularity	0.87 ^a	0.87 ^a	0.86 ^a	0.86 ^a	0.88 ^a
Aspect Ratio	0.84 ^a	0.85 ^a	0.83 ^a	0.83 ^a	0.83 ^a
Elongation	0.16 ^a	0.16 ^a	0.17 ^a	0.17 ^a	0.17 ^a
Solidity	0.97 ^a	0.97 ^a	0.96 ^b	0.97 ^a	0.98 ^a
Convexity	0.98 ^a	0.98 ^a	0.98 ^a	0.98 ^a	0.98 ^a

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹MPC powder was prepared from spray drying of MPC retentates obtained from ultrafiltration or nanofiltration.

²Feed is an MPC powder of retentate from the ultrafiltration of skim milk; NF22 and NF50 are MPC powders of retentates from the nanofiltration of feed at 22 and 50°C; HCNF22 and HCNF50 are MPC powders of retentates from the combined processing of hydrodynamic cavitation and nanofiltration of feed at 22 and 50°C.

³RDI=Relative Dissolution Index.

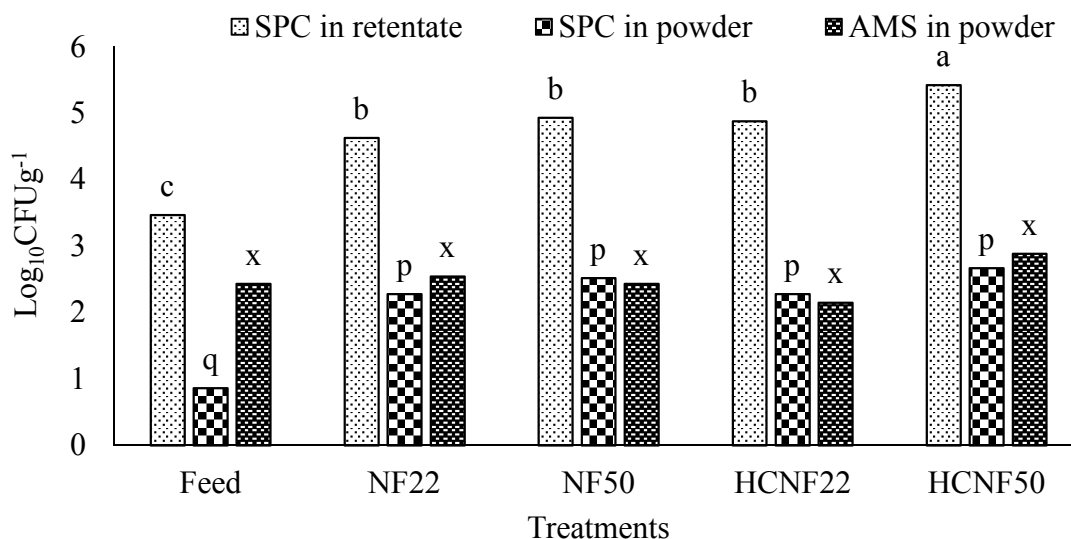


Figure 1. Microbial examination ($\text{Log}_{10}\text{CFUg}^{-1}$, mean, $n=3$). SPC in retentate stands for Standard Plate Counts in the feed and retentates, SPC in powder stands for Standard Plate Counts in the MPC powders dried from feed and retentates, AMS in powder stands for Aerobic Mesophilic Spores in the powders obtained by spray drying of feed and retentates. Feed was an MPC prepared from the ultrafiltration of skim milk; NF22 and NF50 were the retentates from the nanofiltration of feed at 22 and 50°C; HCNF22 and HCNF50 were the retentates from the combined processing of hydrodynamic cavitation and nanofiltration of feed at 22 and 50°C. Values with the same letters (a-c, p-q and x for SPC in retentate, SPC in powder and AMS in powder respectively) on the bars are not significantly different ($P > 0.05$) across all treatments.

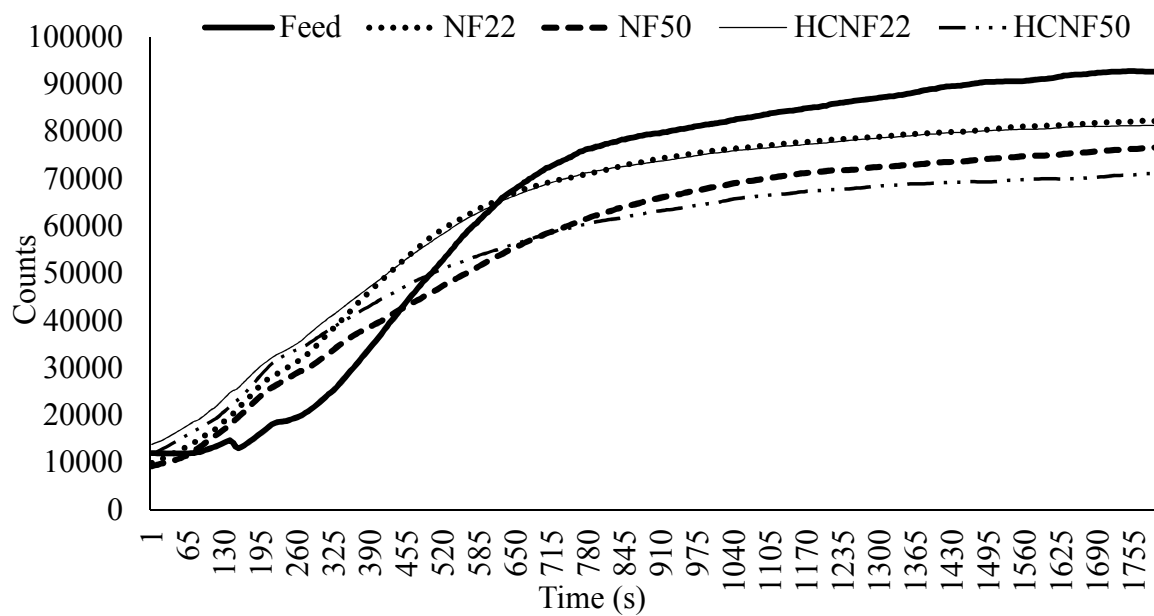


Figure 2. Changes in fine (<10 μ m) counts obtained from the data collected with the focused beam reflectance measurement for MPC powders of different treatments.

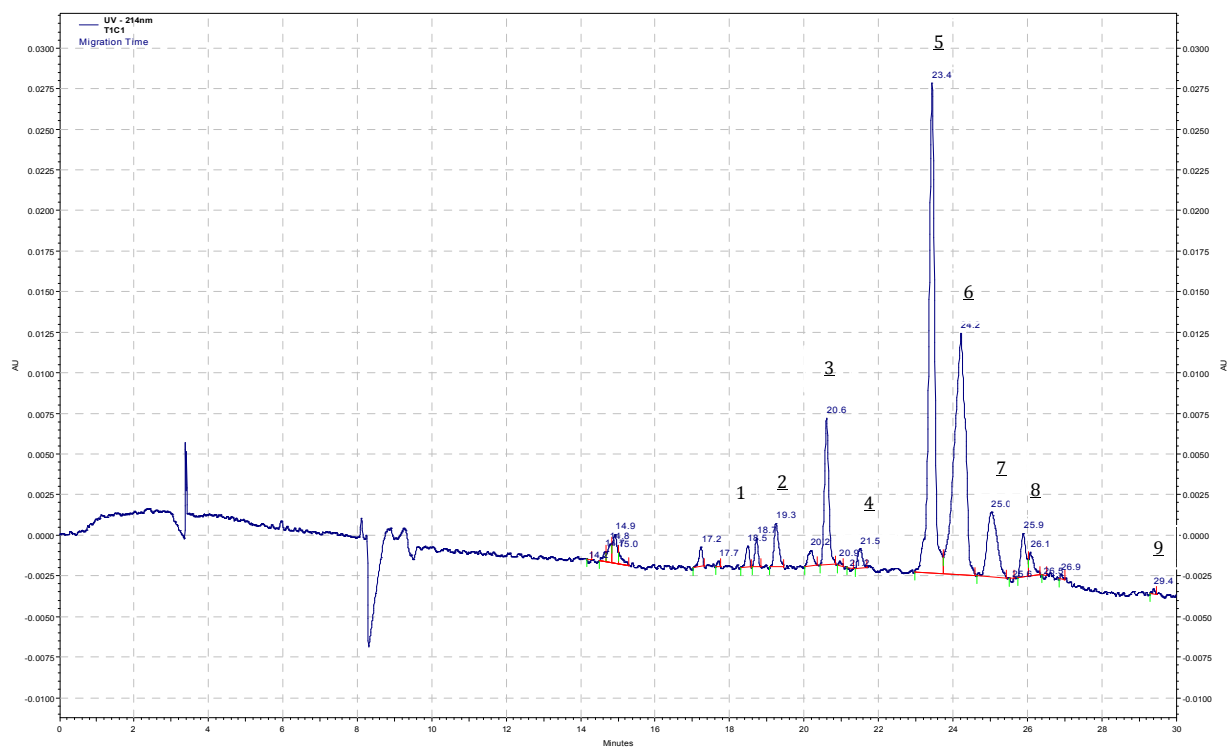


Figure 3. The example capillary gel electrophoresis (CE) electrophoretogram of MPC80. 1=low molecular weight peptides (LMWP); 2= α -lactalbumin (α -LA); 3= β -lactoglobulin (β -LG); 4= γ -casein (γ -CN), 5= β -casein (β -CN); 6= α_{S1} -casein (α_{S1} -CN); 7= α_{S2} -casein (α_{S2} -CN); 8= κ -casein (κ -CN); 9 = high molecular weight peptides (HMWP).

CHAPTER 3

Effect of Cavitation and Nanofiltration Temperature on the Functionality of Milk Protein Concentrate (MPC80)

INTRODUCTION

Milk Protein Concentrate (MPC) is a valuable dairy ingredient to produce a variety of food products. MPC contains highly balanced casein (CN) and whey protein (WP) and shows prominent functional roles. MPCs contain 42 to 85% protein and the CN to WP ratio is similar to skim milk (Patel et al., 2014). MPC80 (80% protein) is produced by ultrafiltration (UF) of skim milk in conjunction with diafiltration to get total solids (TS) of 20% (Marella et al., 2015). To get higher TS before spray drying, MPC80 is further concentrated by using nanofiltration (NF). Concentration polarization and higher viscosity are common problems associated with the NF during manufacturing of high solids MPC (Bacchin et al., 2006). So, in the related research, we had studied the application of hydrodynamic cavitation (HC) and high temperature (50°C) to increase NF efficiency in MPC80 processing.

Hydrodynamic cavitation has been used in various food applications, such as homogenization, mixing, extraction, disinfection, downstream processing, etc. (Asaithambi et al., 2019, Panda et al., 2020) and showed a huge impact on viscosity reduction of MPC fluid and functionality of MPC enriched dairy products (Meletharayil et al., 2016, Li et al., 2018). Before enrichment, the knowledge of flowability matters in MPC powder handling and storage (Fitzpatrick et al., 2017). Most of the functional properties of MPC are relatively similar to the calcium caseinate and not as good as sodium caseinate and WP concentrate (Singh, 2011). Solubility is an important functional

property of food ingredients. Increasing protein level, higher drying and storage temperatures, longer storage time and lower rehydration temperatures reduce the solubility of MPC powder (Udabage et al., 2012), but can be improved by high shear treatment, ultrasound, static high pressure, etc. during MPC production (Augustin et al., 2012, Udabage et al., 2012, Yanjun et al., 2014). Solubility can also be predicted from the wettability, a property of powder which is highly influenced by the particle surface composition, density and other morphological properties (Fitzpatrick et al., 2017). Emulsifying capacity of a protein depends on the particle size of the emulsion droplets generated at certain protein strength under defined homogenization conditions (Singh and Ye, 2020). Foaming capacity of a milk product is the ability of entrapping air bubbles by milk components mainly proteins at specific manufacturing temperature (Kamath et al., 2008). Foamability of milk or milk products can be increased by the heat-induced association of WP with the CN on the surface of homogenized milk fat globules (Huppertz, 2010). Rheology of a dairy ingredient mainly depends on the composition of protein gel matrix and their deformation after rehydration (Vélez-Ruiz et al., 1997). In a rheological test of 10% reconstituted MPC60, Meena et al., (2017) observed a pseudoplastic behavior. Heat stability is another essential property of dairy ingredient in which the ability to withstand high processing temperature is tested at a specified pH (Lehmann and Buckin, 2005). Native WP remain undenatured in a pH of 4.2 to 5.1 but undergo precipitation if it is heat-denatured (Patel et al., 2007) and assumed as a quality index of dairy powders.

Though MPC is a potential ingredient in cheese production, high protein MPC (protein content >70%) are limitedly used in standard cheese making. Mostly, MPC is commonly

used in baker's cheese, processed cheese, and cheese spreads (Patel et al., 2014). To use in cheese, MPC should have proper renneting properties such as rennet coagulation time (RCT), curd firmness, and yield (Rehman et al., 2003). High protein MPC may have a very low level of ionic calcium which elongates renneting time. Externally added ionic calcium improves rennetability of cheese milk (Scott et al., 1998). In this study, we aimed to assess the impact of HC and high-temperature NF on the functional properties of MPC80 powders.

MATERIALS AND METHODS

Experimental Design

Three replicates of MPC having 20% TS and 80% protein (MPC80) based on TS were obtained from UF of skim milk, which were further concentrated using NF.

Hydrodynamic cavitation was applied as a pretreatment, then NF was carried out at two specific temperatures: room temperature (22°C), and high temperature (50°C). Four different NF treatments were utilized including NF at 22°C (NF22); NF at 50°C (NF50); HC prior to NF at 22°C (HCNF22); and HC prior to NF at 50°C (HCNF50). The retentates from NF were spray-dried to get the MPC80 powders which were tested for their functional properties.

Hydrodynamic Cavitation

For the HCNF22 and HCNF50 treatments, the untreated MPC80 was passed once through an APV Cavitor (SPX Flow Technology, Denmark; model CAV8000) set at 50 Hz, a product flow rate of 100 Lh⁻¹, and a pressure of 13.8- to 17.2 kPa. The average inlet and outlet temperatures of MPC80 used for the cavitation were 7 and 34°C, respectively.

For the HCNF22 treatment, the cavitated milk was cooled immediately to room temperature by passing through the heat exchanger system.

Nanofiltration

The feed material was preheated to 22°C for NF22 and HCNF22 treatments and 50°C for NF50 and HCNF50 treatments in a hot water bath. Preheated MPC were then filtered immediately using a spiral wound NF membrane with a molecular weight cut-off 200 Da at respective preheating temperatures until getting the highest level of solids in the retentate.

Spray Drying

NF retentates from different treatments were dried in a pilot-scale dryer (Niro dryer Model 1, Niro Inc., Columbia) having an external mix air atomizing nozzle (Spraying Systems Co., model SUE18A) system. A heat exchanger was set at 50°C in the flowing system to maintain the uniform flow of concentrate. Each retentate was dried to get 2 Kg of powder. The inlet and outlet temperatures used for the drying were set at 175 and 85°C, respectively. MPC powders collected from the bottom of the dryer (heavier particles) and cyclone separator (lighter particles) were mixed proportionally and stored at room temperature at airtight containers. Part of the powder sample was stored at 4°C in airtight containers for further analyses. MPC80 powders were then tested immediately for their functional characterization.

Rennet Coagulation Time

The time for the onset of coagulation with rennet is considered as rennet coagulation time (RCT). Total four sets of each 200 mL reconstituted MPC80 solution containing

3.5% protein based on moisture and protein content on the MPC80 powder from different treatments were prepared in deionized water and stirred using a magnetic stirrer (700 rpm) for 30 minutes at 22°C. Calcium chloride (CaCl_2 , Sigma- Aldrich, assay $\geq 99\%$) was added at the rate of 0.05, 0.1 and 0.25% wt/wt in the final concentration for the three sets of solution and agitated for 5 more minutes followed by pH adjusted to 6.5 using dilute lactic acid. A 60 mL of prepared solution was poured into the cup of rheometer (MCR 92, Anton Paar Ltd., UK) fitted with bob-and-cup configuration at constant strain (0.5%), frequency (1 Hz) and temperature 32°C. 9 μl rennet (CHY-MAX® Extra, CHR HANSEN, material#73812, activity: ~ 650 IMCU/ml) was added, mixed properly and a strain (γ) sweep test was carried out until the storage modulus (G') just crossed 1 Pa. The RCT was defined as the time in a minute when the aggregated system had a storage modulus equal to 1 Pa (Lucey, 2002; Zoon et al., 1988). Pasteurized skim milk (protein content $\sim 3.5\%$) was used as a reference sample to compare the RCT of reconstituted MPC80 solution and a triplicate test was carried out at similar CaCl_2 concentrations at the same testing conditions.

Whey Protein and Denatured Protein

Part of WP can be denatured at high processing temperature and level of denaturation depend on the temperature and extent of heating. WP was determined from the difference of non-CN nitrogen (NCN) and non-protein nitrogen (NPN) (Dupont et al., 2011) of the final retentates from NF treatments before drying as mentioned in table 1. The value obtained from the difference was multiplied by the factor 6.38 to calculate the amount of WP and expressed based on true protein (TP).

Denatured protein formed due to various heat applications in the MPC80 powder manufacturing process was estimated by Kjeldahl method. The sample was processed as described by Morr (1985). In brief, 5 g of MPC80 powder sample was mixed with 60 mL deionized water in a 150mL conical flask and then stirred for 10 minutes at 22°C with the help of magnetic stirrer (700 rpm). The pH was adjusted to 4.6±0.05 using 0.1M hydrochloric acid and continuously stirred for another 30 minutes. The solution was transferred to a 100 mL volumetric flask and diluted to the mark with deionized water and mixed properly. The sample was taken for the estimation of total nitrogen (TN). Further, 45ml of TN-sample was poured to a 50 ml centrifuge tube and centrifuged at 3000 rpm for 20 minutes. The supernatant was filtered through a Whatman™ #1 filter paper (GE Healthcare, UK) and the filtrate was used for the estimation of soluble nitrogen (SN). Nitrogen contents from both TN- and SN- samples were determined by Kjeldahl method and the denatured protein was calculated using equation 1.

$$\text{Denatured protein (\%)} = 100 - \left(\frac{\text{SN}}{\text{TN}} \times 100 \right) \quad [1]$$

Rheological property

The workability of MPC80 powder was studied in terms of rheological property. Reconstituted MPC80 (10% wt/wt) in deionized water was stirred for 30 minutes at 22°C. After holding for 1 minute, 60 mL of MPC80 solution was transferred to the cup of rheometer (MCR 92, Anton Paar Ltd., UK, fitted with bob-and-cup configuration) and the flow behavior was observed at temperature 22°C and a shear rate of 1 to 1000 s⁻¹. Trendline plotted for shear stress versus shear rate was best fitted to Herschel-Bulkley model as described by equation 2.

$$\tau = \tau_0 + K (\dot{\gamma})^n \quad [2]$$

Where σ_0 is the yield stress, σ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency index ($Pa.s^n$) and n is the flow behavior index

Solubility

The solubility of MPC80 powder was assessed as the method described by Haque et al. (2012) with a slight modification. Each 200 mL, 5% (wt/wt) reconstituted MPC80 bulk solution was prepared in deionized water and stirred continuously for 30 minutes using a magnetic stirrer (700 rpm) at 22 and 50°C. Each 40 mL homogenous mixture from bulk solution was transferred to three 50 mL centrifuge tubes and centrifuged at 700xg for 10 minutes at 22°C. The supernatant was transferred carefully in dry pre-weighed aluminum bowl and dried overnight at $103 \pm 2^\circ C$, cooled, and weighed. The solubility of MPC powder was calculated using equation 3.

$$\text{Solubility (\%)} = \frac{\text{dry matter in supernatant}}{\text{dry matter in bulk solution}} \times 100 \quad [3]$$

Foaming Capacity and Foam Stability

Foaming capacity was evaluated as the method described by Shilpashree et al., (2015). A 3g MPC80 powder was blended with 100 mL phosphate buffer (0.05 molL^{-1} , pH 7) in a mixer (auto-mix Osterizer blender, Model: 6630) and whipped for 6 minutes at the speed 11,000 rpm. The developed foam was immediately transferred into 250 mL measuring cylinder quantitatively and total volume was recorded. The foaming capacity was calculated using equation 4.

$$\text{Foaming capacity (\%)} = \frac{\text{Foam volume after whipping} - \text{Liquid volume before whipping}}{\text{Liquid volume before whipping}} \times 100 \quad [4]$$

The cylinder containing foam was kept undisturbed for 30 min at 22°C and then final volume of foam was recorded. The foam stability was determined using equation 5.

$$\text{Foaming capacity (\%)} = \frac{\text{Volume of foam after 30 minutes}}{\text{Initial volume of foam}} \times 100 \quad [5]$$

Emulsifying Capacity, Emulsion Stability and Oil separation

Dispersion of MPC (1%, wt/wt) was prepared by adding MPC80 powder in deionized water and then stirred using a magnetic stirrer (700rpm) for 60 min at 22°C. The pH of the dispersions was adjusted in the range of 6.8 to 7.0 using NaOH. 7g of reconstituted MPC was measured in a 50mL centrifuge tube and then 3g soybean oil was added on that. The mixture of MPC solution and oil was heated to 55°C and homogenized for 60 s at 10,000 rpm using a benchtop homogenizer (Polytron, PT 2500E). Approximately 8 g of the emulsion was transferred to another 15 mL centrifuge, centrifuged at 1100xg for 5 min and the height of the emulsified layer was recorded. The emulsifying capacity was calculated using equation 6.

$$\text{Emulsifying capacity (\%)} = \frac{\text{Height of emulsified layer (mL)}}{\text{Height of total content in the tube (mL)}} \times 100 \quad [6]$$

The emulsion was heated at 80°C water bath for 30 min and cooled to room temperature (22°C) and recentrifuged at 1100xg for 5 min. The emulsion stability was calculated using equation 7.

$$\text{Emulsion stability (\%)} = \frac{\text{Height of emulsified layer after heating (mL)}}{\text{Height of total content in the tube (mL)}} \times 100 \quad [7]$$

Oil separation from the emulsion was measured after 24 h, 7 d, and 90 d of refrigeration storage. Separated oil on the top was collected by using a micropipette and weighed. The oil separation percentage was calculated using equation 8.

$$\text{Oil separation (\%)} = \frac{\text{Weight of separated oil}}{\text{Weight of total oil used in the emulsion}} \times 100 \quad [8]$$

Physicochemical Properties

Heat Coagulation time. Thermal stability of reconstituted MPC80 was estimated in terms of HCT, as described by IDF, 1995 with some modification by Dissanayake and Vasiljevic (2009). A 30% (wt/wt) reconstituted MPC80 solution (pH ~ 6.8) was prepared in warmed (50°C) deionized water and stirred continuously for 30 minutes using a magnetic stirrer (700 rpm) at 50°C. Sample preparation: prepare a MPC80 solution of 30% TS with warm deionized water and stir for 30 minutes at 50°C. A 3g of solution was weighed in a glass vile (8 ml autoclavable E-C vial, Wheaton), and closed air-tight with a screw cap was clamped on the rocker mounted in the oil-bath (vessel containing food-grade mineral oil and heated at 120°C). The time observed for the first coagulation/curd formation of reconstituted MPC observed inside the rocking vial submerged in the hot oil was recorded as the heat coagulation time.

Wettability. The wettability of MPC80 powder was determined in terms of wetting rate at a specified temperature as the method described by IDF (1976) standard 87 with some modification. A 5g powder was poured from a funnel (made of anti-static material, height 100 mm, lower diameter 40 mm, upper diameter 90 mm) into the beaker (diameter 71 mm, height 115 mm) containing 100 ml deionized water. A sieve was clamped between funnel and beaker to make a uniform fall throughout the surface of water on the beaker. The experiment was conducted at 22 and 50°C. The time in min for complete wetting of the powder was recorded.

Flowability. The free-flowing property of MPC80 powder also known for flowability was estimated from the measurement of angle of friction of MPC80 powders as described by Svarovsky (1987). The powder sample was allowed to fall from a funnel (made of

anti-static material, height 100 mm, lower diameter 40 mm, upper diameter 90 mm) to form a bulk solid cone until the free-flowing powder piled up to the lower tip of the funnel as shown in Figure 5. The perpendicular height of the cone, h , was measured from the lower tip of the funnel to the base of heap and the radius of the circular heap was calculated from the mean estimated diameter ($n=4$) of circular heap. The angle of friction (θ) was calculated using equation 9.

$$\text{Angle of friction } (\theta) = \tan^{-1} \left(\frac{h}{r} \right) \quad [9]$$

Flowability results were also compared with the Carr's index and Hausner ratio which were calculated from the readings of loose and tapped density (Table 1) of MPC80 powders using equation 10 and equation 11, respectively.

$$\text{Carr's index } (\%) = \frac{\text{Tapped density} - \text{Loose density}}{\text{Tapped density}} \times 100 \quad [10]$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Loose density}} \quad [11]$$

Statistical Analysis

The replicated data from the analytical results were analyzed statistically using the Agricolae statistical package for Agricultural Research in R programming language (version 3.5.2), developed by R Core Team (2017). The Tukey's Honest Significant Difference test at a 95% confidence interval was used for deciding the level of significance among the treatment means.

RESULTS AND DISCUSSION

The quality and potential use of milk powder is mainly based on the physicochemical composition of the powder matrix, the water transfer phenomena, and its interaction with matrix components (Schuck et al., 2013). In many food applications, varieties of MPC

are used to achieve the desired ultimate quality of food which is generally associated with the functional properties of MPC powders. The functional properties of MPC powder is highly dependent on the composition mainly the quantity and quality of protein. The composition of MPC80 powder obtained from our related research has exemplified in Table 1. The total protein content was in the range of 78.1 to 79.1% on a dry weight basis and was inversely related to the total ash content. Though the amount of (NPN is low (0.48 to 0.66%) in comparison to total protein, the level was higher in the samples from high-temperature NF treatments. The NCN, which exhibited important functional characteristics of MPC powder, was in the range of 2.81 to 2.87% and showed no differences among the treatments. The MPC80 powders used in this study exhibited no differences in fat and lactose contents but total ash content was slightly higher in NF22 as compared to other treatments. The moisture content of the powders was in the range of 3.18 to 4.00%, which was under the limit ($\leq 5\%$) as stated by USDA (2001) for the non-fat dry milk (spray process) and assumed adequately safe for the storage and further application. We did not observe any significant ($P < 0.05$) treatment effects on the moisture of MPC80 powders as we dried all the NF retentates at similar conditions.

Rennet Coagulation Time

The RCT of reconstituted MPC80 at different calcium chloride (CaCl_2) concentration was determined by strain (γ) sweep test performed in rheometer and the results have been compared with the RCT of pasteurized skim milk at same condition (Table 2). No treatment effects on the calcium content of the MPC80 powder was detected (Table 1), so any change in RCT was fully depended on the externally added CaCl_2 . The RCT without adding CaCl_2 was 5 to 6 times higher (100 to 125 min) compared to skim milk (20 min).

We did not observe any significant ($P > 0.05$) impact of HC and high temperature NF on the RCT of reconstituted MPC80 from different NF treatments in the absence of external CaCl_2 . The RCT was decreased considerably in the presence of external CaCl_2 at different concentration for all the treatments. A significant ($P < 0.05$) impact of high temperature NF in the RCT was observed at both 0.05 and 0.1% CaCl_2 concentrations. The range of RCT was 39 to 40 min and 49 to 52 min for the low temperature- and high temperature NF treatments, respectively at 0.05% CaCl_2 concentration. Similarly, the RCT was decreased to 13 and 14 min for the NF22 and HCNF22 treatments and 18- and 21 min for the NF50 and HCNF50 treatments, respectively at 0.1% CaCl_2 concentration. At higher CaCl_2 concentration (0.25%), the range of RCT was 4 to 6 min for all the treatments having no significant ($P > 0.05$) differences among the treatments. Skim milk was coagulated within a minute in the presence of external CaCl_2 ($\geq 0.1\%$). From the results, it can be claimed that a dose of 0.05 to 0.1% (wt/wt) CaCl_2 can be used for the appropriate renneting action of reconstituted MPC80 solution containing 3.5% protein when compared with the skim milk. Externally added CaCl_2 helps both in increasing ionic calcium concentration and decreasing the pH of the cheese milk which accelerates the protein aggregation rate (Wolfschoon-Pombo, 1997). The increased ionic calcium also supports the proteolysis activity of $\kappa\text{-CN}$, which improves renneting efficiency (Fox et al., 2004). Similar past research conducted by using a varying proportion of CaCl_2 in semi-fat milk showed that the process of coagulation started and ended faster with increasing the dose of CaCl_2 which agreed to our results (Landfeld et al., 2002). Another study conducted for the RCT test in medium- and high-heat reconstituted milk (12%) at 0% and 0.07% CaCl_2 and 0.2 g Kg^{-1} rennet also showed similar results (Klandar et al.,

2007), where RCT of low- and high-heat milk samples were comparable to our samples from low- and high-temperature NF treatments. As stated by Ferrer et al., (2008), when the level protein increases in the MPC powder the release of CN macropeptide would be lowered during renneting which results in longer coagulation time (>60 min). In the same study, they also mentioned that the ionic equilibrium (mainly calcium ions) plays the key role in the renneting behavior of reconstituted MPC. There is a cumulative effect of heat treatment on the renneting activity of protein. During heating of milk, the formation of heat-induced protein complex of WP (β -LG and α -LA) and κ -CN with the help of disulfide bond can be increased which may takes longer time for the rennet digestion (Singh et al., 1988). In the current study, there was an adequate heat exposure to form the whey to κ -CN complex especially in the case of high-temperature NF treatments, which could be the reason for longer RCT as stated in previous studies. The increased level of denatured protein in the high-temperature NF treatments in our studies (Table 3) also supports the possible longer RCT for the samples from high-temperature NF treatments.

Whey Protein and Denatured Protein

Whey proteins play a key role in functionality, are susceptible to the heat treatments, and can be denatured or form complexes with CN at high temperatures and the degree of denaturation depends on the extent of heating. As described by Patel et al, (2014), MPC contains CN to WP ratio likely in skim milk that is ~80% CN and ~20% WP based on the true protein content. WP content in the MPC80 powder from our studies was also closer to the abovementioned value (Table 3). Both HC and high temperatures did not have a significant impact on the WP retention during NF however it was slightly lower in the samples from high-temperature NF though it was statistically insignificant ($P > 0.05$). We

had estimated WP contents from the difference of NCN and NPN. At high temperature, WP, which are soluble even at low pH, can bind with κ -CN and can be precipitated at pH 4.6 and accounted as insoluble CN fractions (Walstra and Jenness, 1984) during the estimation of NCN (Zhang and Metzger, 2011). Whey protein composed of two major fractions, β -LG (~50%) and α -LA (~20%) and minor fractions including bovine serum albumin, immunoglobulins, lactoferrin and proteose peptones and the denaturation of these proteins can be influenced by concentration, pH, total solids, method and intensity of heat treatment, and mineral content (Brown, 1988, Anema et al., 2003). Oldfield et al, (2005) mentioned that preheating bovine milk at 70°C for 52 Sec showed the reduction of WP fractions such as α -LA, β -LG, Immunoglobulins and bovine serum albumin by 30, 20, 20 and 15% respectively. Unlike that high temperature, we preheated the milk at 50°C and NF at the same temperature for the NF50 and HCNF50 treatments also showed slight impact on the WP contents of MPC80 powders.

Similarly, denatured protein content in MPC80 powder was also estimated and presented in Table 3. The level of denatured protein in the NF22 treatment was low, 0.44% based on total protein. It was significantly higher ($P < 0.05$) in rest of the treatments. These results verified that there was a significant level of protein denaturation (7.27 to 8.32%) at high-temperature NF treatments. Our results depicted that HC also contributed significantly ($P < 0.05$) in the protein denaturation which might be due to moderate heat developed by the shearing action of HC and the level of denaturation increased in the combination of HC and high-temperature NF. Limited past research are available on the impact of HC on the MPC; however, a similar trend was observed in HC treated skim milk concentrate studied by Dahiya (2016). One of the studies on the effect

of HC on the properties of a protein-rich low-fat yogurt showed that when the intensity of shock wave reactor increased during cavitation there was a larger particle size which can be possible by the re-aggregation of denatured proteins after the pressure relief (Chaudemanche, 2016), which is similar to our results.

Rheological Properties

We observed the rheological behavior of reconstituted MPC80 (10% wt/wt) and recorded for the apparent viscosity, yield stress, consistency index, and flow behavior index and results are displayed in Table 4. Results showed that both HC and high temperature impacted significantly ($P < 0.05$) on the reduction of viscosity of reconstituted MPC80. The NF22 had significantly ($P < 0.05$) higher viscosity (19 cP) and the viscosity of HC treated samples was in narrow range (12 to 13 cP) with no significant ($P > 0.05$) difference. The higher viscosity of NF22 samples might be due to the higher total minerals (Table 1) as well as ionic calcium contents as compare to other treatments. Smaller particle size because of HC and lower available minerals and ionic calcium might be correlated to the lower apparent viscosity in the HC treated samples. Similar findings were made by Meena et al., (2017) on their study of MPC60 with homogenization. When we observed the rheological behavior of reconstituted MPC, the trend line of shear rate versus shear stress showed Bingham pseudoplastic behavior best fitted with the Herschel-Bulkley model with their coefficient of determination (R^2) value ≥ 98 . The parameters for the Herschel-Bulkley model has shown in Table 4. Similar flow behavior results have been reported in a study conducted for the 10 to 16% reconstituted sodium caseinate solution (Fichtali et al., 1993).

Both HC and high temperature impacted the yield stress. Sample from HCNF50 treatment had the lowest yield stress (1.38 Pa) whereas it was significantly ($P < 0.05$) higher for the sample from NF22 treatment (2.11 Pa). Similarly, both HC and high-temperature NF showed a significant impact on the consistency index as well as the flow behavior index. The consistency index was significantly ($P < 0.05$) lower for the HC treated samples but their flow behavior index was higher ($P < 0.05$). Reconstituted MPC80 solution without HC exhibited more consistent gel but their flowing behavior was poorer whereas the HC treated samples showed better flowing behavior though they displayed less consistency. Similar information was observed by Arzeni et al., (2012) when ultrasonication (20 kHz) was applied for 20 min on the WP, soy protein, and egg white. The consistency index of a food fluid can directly be correlated to the viscosity of the fluid of similar flow behavior index (n) (Doublier and Lefebvre, 1989). It tends to be shear thinning when $n \leq 1$ and shear thickening when $n > 1$. When the flow behavior index is close to 1 the fluid's behavior tends to pass from shear-thinning to a shear thickening (Pevero et al., 2007), this information supported to describe the flow behavior of reconstituted MPC80 used in our study. Unlike thixotropic fluids, when the reconstituted MPC80 solutions exposed at the shear rate 1 to 1000 s^{-1} the viscosity was decreased in the beginning and gradually increased after some time. This nature of shear-thinning followed by shear-thickening actions might be due to structural changes of protein matrix in the MPC80 solution with increasing shear rate. This type of non-linear with a positive yield stress flow behavior supported to categorize the MPC80 solution as a non-Newtonian time-independent fluid, however, it can be varied with concentration and temperature of the MPC fluid. O'Donnell and Butler (1999) reported a shear-thinning

behavior of 20% reconstituted MPC85 at 20°C and the effect of shear was adequately described by the power-law model, which was comparable to our findings for the NF22 and NF50 treatments as we assumed that Herschel-Bulkley model is a modified power-law model.

Solubility

The solubility, also known as colloidal dispersibility, of MPC80 powders was determined at two different temperatures; 22 and 50°C in deionized water and result has presented in Figure 1. The solubility of MPC80 powders was increased from 14 to 16% at a higher dissolving temperature (50°C). The range of solubility was 70.03 to 79.20% and 86.05 to 92.91% when measured at 22 and 50°C, respectively. The variation in the solubility was less when dissolved at 50°C water compared to 22°C. As described in the previous studies (Mistry, 2002, Mimouni et al., 2009), our results also agreed that solubility of MPC powder highly depends on reconstitution temperature. It can be described that the kinetic energy from hot water more effectively breaks the MPC powder particles and dissolve in a better way. The solubility of powder samples from NF22 and HCNF22 treatments are comparable to the findings of similar studies by Rupp et al., (2018). The solubility of MPC80 powders from high-temperature NF treatments was significantly ($P < 0.05$) lower at both 22°C and 50°C while HC did not show any impacts on the solubility at both temperatures. Li et al., (2018) also mentioned that there was not any impact of HC on the solubility of MPC80 powder when applied on the retentates before spray drying. They also reported that they observed lower apparent viscosity when measured immediately after HC treatment and that could not be correlated with the solubility of MPC80 powder after drying. Meena et al., (2017) also reported increased

solubility when observed in homogenized MPC60 powder which can be compared to our HC treatments. They also claimed that the shearing action in milk processing could improve the solubility. Similar findings were also made by Sikand et al., (2011), which were agreed to our findings.

Some of the past studies described the reasons for decreased solubility for the MPC powders processed at higher temperatures (Anema et al., 2003, Huppertz et al., 2018). Another study explained when milk processed between 4 and 60°C, a reversible physicochemical change such as hydrophobic association and partial unfolding can be observed as changes of solubility to some extent (DeWit and Klarenbeek, 1984). We had achieved high solids in the high-temperature NF retentates which eventually led to higher viscosity, which also might alter the composition of protein-matrix and showed negative impacts on the solubility of MPC80 powders from high-temperature NF treatments. Similar kinds of information have been reported in high protein commercial spray-dried MPCs (De Castro-Morel and Harper, 2002). Casein micelles of high protein MPC powders may take several hours to release CN from powder particles in the solution at lower temperatures and moderate agitation conditions (Schuck et al., 2013). For the appropriate rehydration, MPC needs to rehydrate with high shear rates at higher temperatures (Havea, 2006, Mimouni et al., 2010), which might be the reason for the lesser solubility of MPC80 powders from high-temperature NF treatments when dissolved in 22°C water.

Foamability

The presence of discrete gas or bubble phase dispersed in a continuous phase (either solid or liquid) is known as foam. In the food system, protein plays a crucial role in

developing and stabilizing foam. Foaming of milk powder is the interfacial property of their particles at water interface (Uluko et al., 2016). Milk proteins (both CN and WP) have a strong tendency to adsorb at air-water interface to keep foams against various physicochemical instability (Dickinson, 2003). Our results showed that all MPC80 powders were of good foaming capacity and foam stability. The foaming capacity of the MPC80 powders was in the range of 92.22 to 112.89% when measured at neutral pH at ambient temperature (22°C). The stability of the foam was measured after 30 min based on the initial foam volume and was found in the range of 95.89 to 96.68%. Both foaming capacity and foam stability of MPC80 powders are shown in Figure 2. Though the foaming capacity of MPC80 powders was statistically similar ($P > 0.05$) among the treatments, slightly higher foaming was observed in HC treated samples. But in contrary to that, the stability of the foam was slightly lower in the same samples through the readings were not a significant ($P > 0.05$) differences among the treatments.

During foaming, air along with water vapor is forced into a protein solution. The proteins generate a spherical area around the air and stabilize into bubbles. After foaming, the polar nature of milk proteins attract water at one end oriented towards bubble whereas it repels in another end which helps to keep the bubble unbroken (Marinova et al., 2009). Moreover, CN does not have internal covalent crosslinks and does not show any tendency to polymerize through intermolecular disulfide bonds and exhibited distinct amphiphilic nature because of having a highly non-uniform distribution of hydrophilic and hydrophobic residues and acts as a water-soluble surfactant (Leclerc and Calmettes, 1997). Casein undergo reversible self-association in water due to its hydrophobic interactions and this property increases with temperature and ionic strength

(Schmidt et al., 1972). In our studies, most of the proteins in MPC80 powders composed of CN which might be the reason for good foaming capacity. When milk processed between 60-100°C, WP denaturation would be irreversible which helps in improving the foaming property but can be changed their stability at small changes in pH (deWit and Klarenbeek, 1984). The foaming capacity of MPC powder also related directly to the solubility, protein concentration, level of denaturation, and pH of the medium (Mistry and Hassan, 1991). As our MPC80 powders were prepared by spray drying with the outlet air temperature 85 to 90°C, both foaming capacity and their stability were nearly double when measured at neutral pH. Uluko et al., (2016) also mentioned that foaming stability depends on the characteristics of WP available in MPC powder. In the study conducted by Marinova et al., (2009), the foaming capacity and the stability of sodium caseinate was very good at neutral pH, as the concentration of CN in MPC80 powder is also high which can be correlated to our results. Foaming properties of MPC80 powders made from different concentration methods such as ultrafiltration/diafiltration and or evaporation method were similar as their final protein content was similar to each other (Rupp et al., 2018), which is agreed to our result. In comparison to other food products, the research in foaming properties of MPC has gotten less interest. Past studies related to the ultrasonication on the WP concentrate at 20kHz increased the surface hydrophobicity and thereby increased foaming properties (Arzeni et al., 2012). Similar results were observed in the giant squid mantle proteins (Arredondo-Parada et al., 2020) in which researchers claimed that effect of sonication reduced the particle size as well as three-dimensional structure of the proteins and exposed more hydrophobic groups to the

surface resulted in better foaming properties. These findings agreed to the results of our HC treated samples.

Emulsifying Capacity, Emulsion Stability and Oil separation

Results of emulsifying capacity and emulsion stability are demonstrated in Figure 3. The emulsifying capacity of MPC80 emulsions with sunflower oil (30% wt/wt) measured at ambient temperature was in the range of 59.58 to 61.38% based on total volume. An accelerated test of emulsion stability carried out by heating the emulsion at 80°C for 30 minutes showed the reduction in emulsion volume by 15% in an average, hence the emulsion stability was in the range of 45.49 to 47.28%. MPC80 powders from both HC and high-temperature NF treatments showed slightly higher emulsifying capacity when compared to NF22, however, the difference was not a significant ($P > 0.05$). Similarly, samples from HC/NF22 treatment exhibited little better emulsion stability but the difference to other treatments was statistically insignificant ($P > 0.05$). Research related to the impact of HC on the emulsifying capacity and emulsion stability is very limited however we compared with similar research findings from ultrasonication and homogenization. Meena et al., (2017) reported slightly lower emulsifying capacity (37.62 to 43.50%) of MPC60 powder, but they observed less reduction in emulsion volume after the emulsion stability test. The higher emulsifying capacity in our samples might be due to the higher protein content.

The chemistry of protein-emulsion (droplets) is very similar to the protein-foam (bubbles). The emulsion can be converted to foam when the shape of droplets changed from rounded globule to faceted polyhedral (Blauer et al., 1988). In either property, proteins act as surfactants. Both CN and WP of milk reveal strong inclination to adsorb at

oil-water and air-water interfaces which play vital roles to form the emulsion/foam and provide protection against the physicochemical forces for their longer existence (Dickinson, 2003). Soluble proteins easily migrate towards the surface of bubbles during emulsification, so, emulsification capacity can be predicted from the solubility. The effectiveness of interfacial surface activity of milk proteins available in MPC highly dependent on the production process, particle size, and concentration of protein and calcium contents (Ye, 2011). The particle size of HC treated samples are comparatively lower (Table 1). Though statistically insignificant ($P > 0.05$), the better emulsion stability exhibited by the powder sample from HCNF22 treatment agreed to the abovementioned research findings. In comparison to our results, higher emulsion stability (77 to 86%) was observed when tested in an aqueous phase (WP concentrate, 1% solution) to oil phase (flax seed oil) ratio 60:40 including potassium sorbate (0.1%) as a stabilizer (Lee and Choo, 2015), which might be the impact of pure WP and the stabilizer.

Oil separation was measured as a test of emulsion stability when stored at refrigeration temperature. It was measured after 24 h, 7 d, and 90 d of emulsion preparation and the result as a percentage of oil separation based on the amount of oil used shown in Figure 4. The rate of separation was maximum within 24 h of emulsion preparation. For the first 24 h, about 5% oil was separated from the emulsion and the difference in the separation was in a narrow range (5.20 to 5.40%). Among the results, the separation was lowest for the HCNF22 treatment and highest for the NF50 treatment and the difference was statistically significant ($P < 0.05$). The lowest separation which also reflects the better emulsion stability in the emulsion of HCNF22 treatment might be having finer particles and less denatured proteins available in that sample. The separation of oil was increased

continuously on the 7th d (5.70 to 6.29%) and 90th d (7.99 to 8.38%) of storage. The oil separation trend of 24 h, 7 d and 90 d storage period showed that the lowest separation was in HCNF22 treatment and higher separation was in high-temperature NF treatments, however, the differences in the separation was not a significant ($P > 0.05$). Lee and Choo (2015) also found similar results of oil separation when observed in the emulsion of WP concentrate for 7 d. Previous studies showed that the emulsion prepared by using triglycerides are more stable compare to carbohydrate derivatives. Similarly, emulsion having smaller emulsion droplets and a pH higher than the isoelectric point showed less oil separation and better emulsion stability (Ye, 2011, Orciuch et al., 2012).

Physicochemical Properties

Heat Coagulation time. Result of HCT helps to decide for how long and at what temperature the MPC80 powder alone or its fortified product can be processed without altering the properties of protein during food manufacturing. Heat stability of bovine skim milk is measured at 140°C whereas it is measured at 120°C for the concentrated milk because of the increased level of protein (Singh, 2004). We had estimated the HCT of reconstituted MPC80 samples at 120°C using oil bath and found in the range of 18.04 to 22.89 min (Table 5). As the results showed the lowest HCT for the sample from HCNF50 treatment, it can be claimed that both HC and high temperature had a significant ($P < 0.05$) impact on the HCT. As described by Fox and Morrissey (1997), HCT can be influenced by processing temperature, lactation stage, β -LG to κ -CN ratio, colloidal calcium phosphate concentration, and other soluble salts. Caseins tolerate high processing temperature whereas WP are susceptible to heat and can be denatured completely within 10 minutes at 90°C. Higher pH or low level of calcium ions elongates

the heat stability of CN powders due to the buffering activity (Panouillé et al., 2004). The decreased in heat stability were reported in few past studies including; heat and micro-fluidization of WP (Iordache and Jelen, 2003), heat and homogenization in condensed milk (Deyscher et al., 1929) and hydrodynamic cavitation in skim milk concentrate (Dahiya, 2016) are in agreement to the results of current study. It can be claimed that the shorter HCT in the HC and or high-temperature NF treatments might be due to the formation of disrupted WP aggregates (a complex of β -LG and κ -CN) by the action of heating or high shear activities (Dissanayake and Vasiljevic, 2009, Sharma et al., 2012) such as cavitation. These protein aggregates are sensitive to secondary heat-induced coagulation (Iordache and Jelen, 2003) as we conducted during HCT measurement.

Wettability

The wetting property of MPC80 powder in water was observed at two different temperatures 22 and 50°C and recorded as wetting time (min). At 22°C, the range of wetting time was 140-198 min. In comparison to 22°C, wetting time was reduced considerably when observed at 50°C (87-133 min) (Table 5). At both temperatures (22 and 50°C), wetting time was reduced significantly ($P < 0.05$) for the HC treated samples. In a previous wettability study conducted in MPC samples containing 75 and 85% proteins at 20, 50 and 70°C, researcher reported the wetting time more than 1 h for both samples at all temperatures. Wettability was slightly improved when temperature increased from 20 to 50°C but worsened higher than that temperature (Fitzpatrick et al., 2017). Wetting is the first step of rehydration and wettability reflects as the ability of a powder to absorb water at its surface and swell (Alghunaim et al., 2016). Protein powders generally swells and disperse when absorb water (Hussain et al., 2011). In general, dry

milk powders with wetting times more than 60 s are considered non-instant (IDF, 1979); so, MPC powder can be categorized as non-instant. Wettability also depends on the protein content. Previous studies showed that when protein content in MPC increased, the hydrophobicity increased due to the interactions of α - and β -CN at protein-rich surface, which influence the wettability especially when protein content become more than 65% (Fitzpatrick et al., 2017, McSweeney et al., 2020). As described by Sharma et al., (2012), wetting rate of a milk protein powder is affected by particle size, porosity, surface area of the particles. According to Singh and Ye (2010), high-pressure homogenization and or preheating of milk concentrates prior to spray drying results poor reconstitution properties of the milk powder, which can be correlated longer wetting time of MPC powders in our studies. In comparison to other treatments, wetting time was significantly reduced in the samples from HC treatment which might be due to the reduction of particle size thereby increasing the porosity and surface area of the powder where the water could easily be penetrated. Li et al., (2018) mentioned that as other MPC80 powders the wetting time of HC treated MPC80 powders was >300 s, which agreed to our results.

Flowability

Flowability of MPC80 powders was evaluated from three different parameters including angle of friction, Carr's index, and Hausner ratio. Angle of friction is the angle measured between the flat surface and the cone surface of the loose powder when it is poured onto a flat surface. Similarly, the Carr's index (Carr, 1965) and Hausner ratio (Hausner, 1967) of the powder depend on their tapped- and loose bulk densities. Powder samples from all treatments had the narrow range of angle of friction (39.89 to 41.69°),

Carr's index (20.19 to 23.48) and Hausner ratio (1.25 to 1.38). Granulated free flowing powder generally have an angle of repose/friction $\leq 40^\circ$ (Ewsuk, 2001). Results from the angle of friction, Carr's index values and Hausner ratio values showed that the flowability of MPC80 powders was in between of fair (Carr's index; 16 to 20%, Hausner ratio; 1.19 to 1.25) and passable (Carr's index; 21 to 25%, Hausner ratio; 1.26 to 1.34) category. Neither HC nor high temperature impacted significantly ($P > 0.05$) on the flowability of MPC80 powders. However, HC treated samples showed poorer flowability among the treatments. Flowability is highly dependent on the particle size, surface roughness, surface chemical composition and electrostatic charge (Barbosa-Canovas et al. 1987, Kim et al., 2005, Jallo and Dave, 2015). Fine powders having the diameter $< 100\mu\text{m}$ show poor flowability and fluidization because of the cohesive forces (Visser, 1989). Particle size of MPC80 powders used in this study was in the range of 8.93 to $9.51\mu\text{m}$ (Table 1) verified that these powders can be of higher interparticle resistance and can form aggregated particles by adhering each other and thereby lumps resulted poor flowability. As in native condition, MPC powder might have higher surface roughness which accumulates higher surface energy and leads to increase the inter-particle adhesion force and results poor flowability. Recent studies show that use of NaCl or KCl (Sikand et al., 2016) or use of CO_2 (Marella et al., 2015) during MPC production by ultrafiltration process helps in the reduction of calcium level and surface negative charge. These modified MPC after spray drying not only show better rehydration or solubility but also increase the flow properties.

CONCLUSIONS

Effect of HC and high-temperature NF on the various functional properties of MPC80 powders were studied. RCT test showed that MPC80 powders from our studies are capable to form cheese curd by adding CaCl_2 in between 0.05 to 0.1%. The flow behavior of the reconstituted MPC80 was improved with HC. Rheological test of 10% MPC80 solution showed a shear-thinning followed by shear thickening behavior which can be described as non-Newtonian time-independent flow and was best fitted with the Herschel-Bulkley model ($R^2 \geq 98\%$). High-temperature NF reduced the solubility of MPC80 powder at both ambient and warm temperatures. Both HC and high temperatures did not impact the flowability, emulsifying capacity, emulsion stability, and WP retention but improved the wettability of MPC80 powder. Heat stability and the level of denatured protein were affected slightly by the combined action of HC and high-temperature NF. The foaming capacity of MPC80 powder was increased with HC when observed at neutral pH. Overall, the findings of this study showed that HC and NF temperature have important impacts on the functionality of MPC80 powders.

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Table 1. Mean (n=3) compositional analysis of MPC80 powders used in this study

Properties	NF22 ¹	NF50 ¹	HCNF22 ¹	HCNF50 ¹
Moisture (% wt/wt)	4.00 ^a	3.32 ^a	3.96 ^a	3.18 ^a
Total Proteien (% dry basis)	79.10 ^a	78.06 ^a	78.62 ^a	78.10 ^a
Lactose (% dry basis)	9.58 ^a	9.39 ^a	9.34 ^a	9.78 ^a
Total Ash (% dry basis)	8.30 ^a	7.65 ^b	7.55 ^b	7.66 ^b
Crude Fat (% dry basis)	3.35 ^a	3.80 ^a	3.98 ^a	4.01 ^a
Non-casein nitrogen (% dry basis)	2.87 ^a	2.84 ^a	2.81 ^a	2.87 ^a
Non-protein nitrogen (% dry basis)	0.48 ^b	0.66 ^a	0.49 ^b	0.66 ^a
Calcium (mg/100g)	2012 ^a	2228 ^a	2231 ^a	2049 ^a
Loose Density (Kg/m ³)	273 ^a	278 ^a	272 ^a	286 ^a
Tapped Density (Kg/m ³)	344 ^{bc}	348 ^b	356 ^{ab}	366 ^a
Particle diameter (µm)	9.30 ^a	9.51 ^a	8.93 ^a	9.49 ^a

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively.

Table 2. Rennet coagulation time (minutes, mean, n=3) of reconstituted¹ MPC80 at different CaCl₂ concentration

CaCl ₂ (%) wt/wt)	SKM ²	NF22 ³	NF50 ³	HCNF22 ³	HCNF50 ³
0	20	105 ^a	125 ^a	100 ^a	121 ^a
0.05	7	39 ^c	52 ^a	40 ^c	49 ^b
0.1	<1	14 ^c	21 ^a	13 ^c	18 ^b
0.25	<1	4 ^a	6 ^a	4 ^a	5 ^a

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹Reconstituted MPC80 containing 3.5% protein based on moisture and protein content on the MPC80 powder from different treatments.

²Pasteurized skim milk (protein content approximately 3.5% wt/wt).

³Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively.

Table 3. Mean (n=3) whey protein and denatured protein of MPC80 powders

Treatments ¹	Whey Protein ² (%)	Denatured Protein ³ (%)
NF22	19.27 ^a	0.44 ^d
NF50	17.79 ^a	7.27 ^b
HCNF22	18.89 ^a	2.27 ^c
HCNF50	18.05 ^a	8.32 ^a

^{a-d}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively.

²Based on true protein on dry weight basis.

³Based on total protein on dry weight basis.

Table 4. Rheological properties analysis (mean, n=3) of reconstituted¹ MPC80

Treatments ²	Apparent Viscosity ³ (cP)	Parameters for the Herschel-Bulkley model ⁴			
		Yield stress (Pa)	K	n	R ²
NF22	19 ^a	2.11 ^a	0.015 ^a	0.889 ^b	0.98
NF50	14 ^{ab}	1.90 ^a	0.010 ^b	0.994 ^{ab}	0.99
HCNF22	13 ^b	1.59 ^b	0.008 ^{bc}	1.006 ^{ab}	0.99
HCNF50	12 ^b	1.38 ^b	0.005 ^c	1.071 ^a	0.99

^{a-c}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹Reconstituted MPC80 containing 10% total solids (wt/wt).

²Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively.

³Viscosity measured at shear rate 1 to 1000s⁻¹ in bob and cup configuration.

⁴Herschel-Bulkley model; $\bar{\sigma} = \bar{\sigma}_0 + K(\gamma)^n$, where, $\bar{\sigma}_0$ is the yield stress, $\bar{\sigma}$ is the shear stress (Pa), γ is the shear rate (s⁻¹), K is the consistency index (Pa.sⁿ) and n is the flow behavior index.

Table 5. Mean (n=3) physicochemical properties analysis of MPC powders

Treatments ¹	HCT ² (min)	Wetting time at 22°C (min)	Wetting time at 50°C (min)	Flowability test parameters ³		
				Angle of Friction	Carr's Index (%)	Hausner Ratio
NF22	22.89 ^a	186 ^a	128 ^a	41.69 ^a	20.77 ^a	1.27 ^a
NF50	20.22 ^b	198 ^a	133 ^a	41.67 ^a	20.19 ^a	1.25 ^a
HCNF22	19.34 ^b	140 ^b	90 ^b	40.47 ^a	23.48 ^a	1.31 ^a
HCNF50	18.04 ^b	154 ^{ab}	87 ^b	39.89 ^a	21.70 ^a	1.28 ^a

^{a-b}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively.

²Heat coagulation time of reconstituted MPC80 (30% wt/wt) measured at 120°C using oil bath.

³Angle of friction, $\theta = \tan^{-1}(h/r)$; h and r are the height and radius of conical-heap of powders. Carr's index and Hausner ratio are calculated from loose and tapped densities of powders.

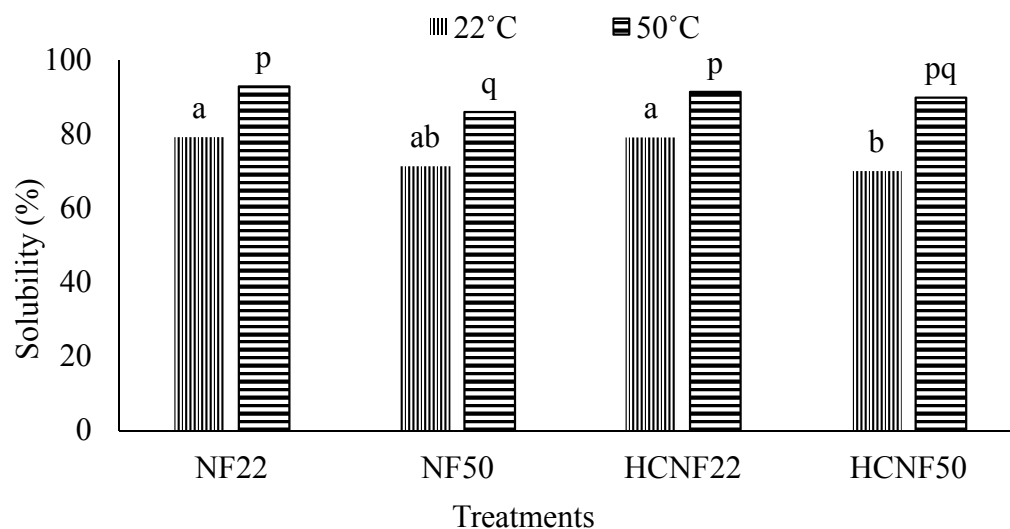


Figure 1. Solubility (%; mean, $n=3$) of MPC80 powders. Solubility was measured in reconstituted MPC80 solution (5% wt/wt) with deionized water at 22 and 50°C. Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively. Values with the same letters (a-b for 22°C and p-q for 50°C bars) are not significantly different ($P > 0.05$) across all treatments.

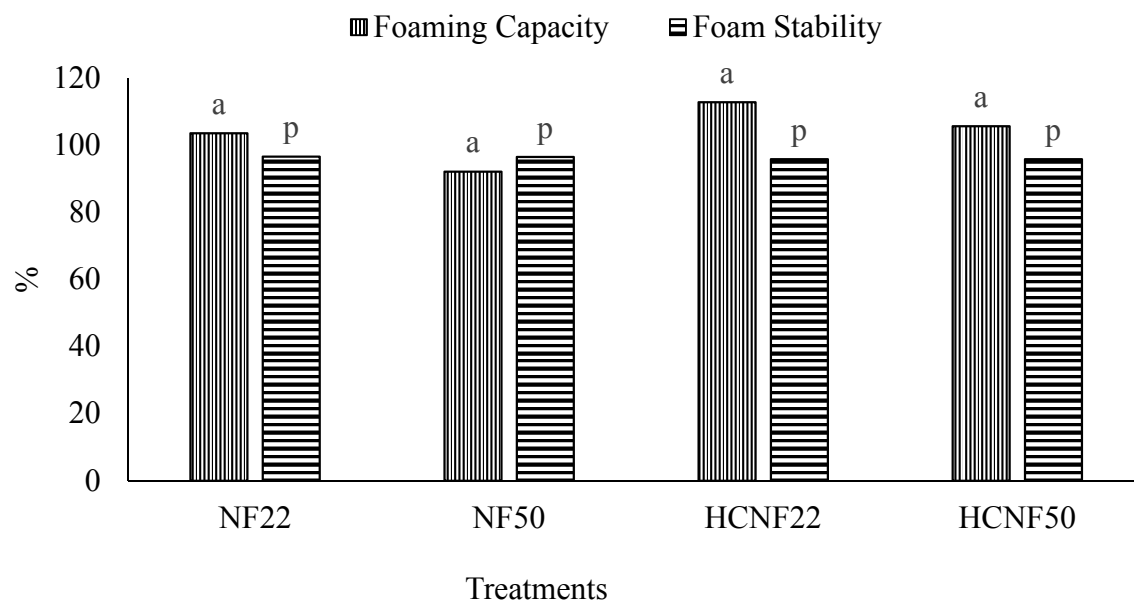


Figure 2. Foaming capacity (% v/v, mean, n=3) and foam stability (% v/v, mean, n=3) of MPC80 powder. Foaming capacity was measured in reconstituted MPC80 solution (3% wt/wt) with phosphate buffer (0.05 mol/L, pH=7.0) at 22°C. Foam stability was observed after 30 minutes. Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively. Values with the same letters on the bars of foaming capacity or foam stability are not significantly different ($P > 0.05$) across all treatments.

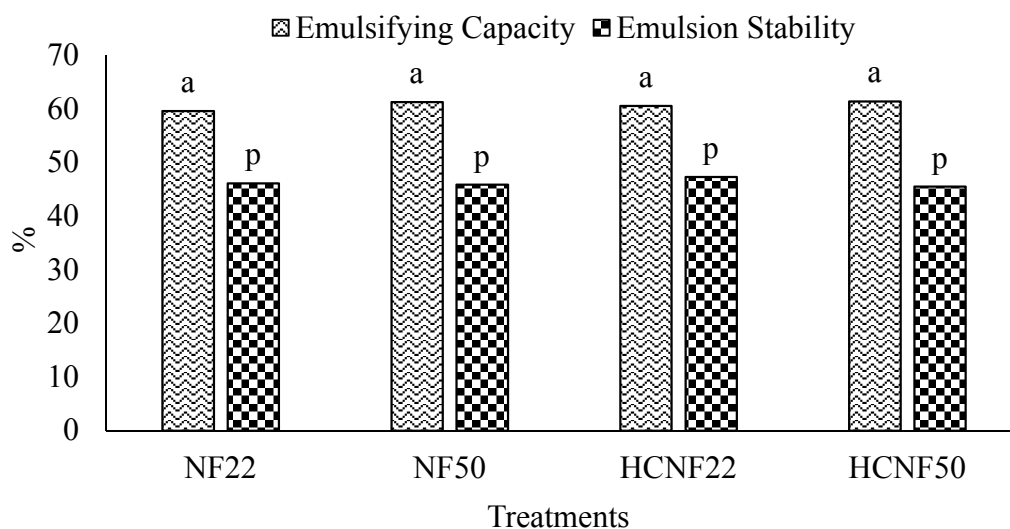


Figure 3. Emulsifying capacity (% , mean, n=3) and emulsion stability (% , mean, n=3) of MPC80 powder. Emulsifying capacity was measured by mixing sunflower oil (30% wt/wt) with the MPC80 solution (1% wt/wt). Emulsion stability was estimated after heating the emulsion at 80°C for 30 minutes. Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively. Values with the same letters on the bars of emulsifying capacity or emulsion stability are not significantly different ($P > 0.05$) across all treatments.

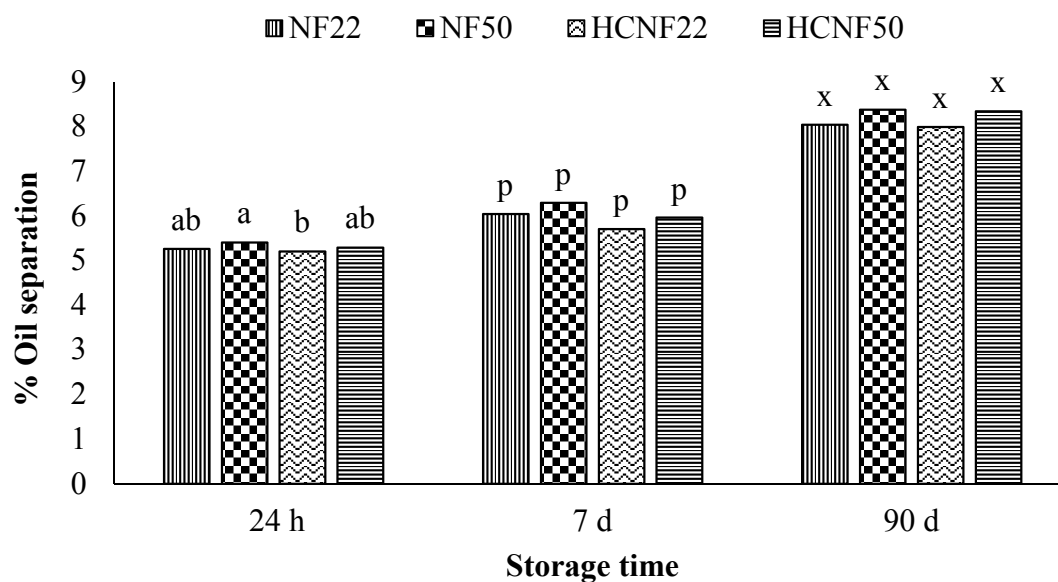


Figure 4. Oil separation (% wt/wt, mean, n=3) from the emulsion of MPC80. Oil separation was measured in the emulsion of MPC80 stored for 24 h, 7 d and 90 d at 4°C based on oil used. Treatments such as NF22 and NF50 were the MPC80 powders from the nanofiltration at 22 and 50°C, respectively, and treatments such as HCNF22 and HCNF50 were the MPC80 powders from the combined processing of hydrodynamic cavitation followed by nanofiltration at 22 and 50°C, respectively. Values with the same letters on the bars of same storage periods are not significantly different ($P > 0.05$) across all treatments.

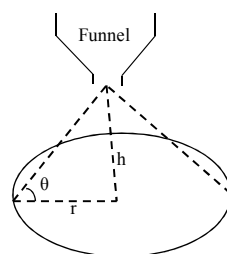


Figure 5. Ideal bulk solid cone assumed to be formed during free flowing of MPC80 powder.

CHAPTER 4

Effect of Temperature on the Performance of Plate and Frame Filtration During Milk Protein Concentrate Manufacture

INTRODUCTION

Membrane technology plays a vital role in dairy processing industries mainly in milk protein concentration to get its concentrate and isolate forms. For the milk protein concentrate (MPC) having protein content >65% in the final retentate, ultrafiltration (UF) is generally utilized in conjunction with diafiltration. During MPC production, the level of total solids (TS) in UF retentate limits to ~20% and further concentration is mostly done by using nanofiltration (NF) system. In NF system, the level of TS of MPC80 achieved ~25% when processed at room temperature (Mishra and Metzger, 2019a; Cao et al., 2015). This limited TS might be due to increasing apparent viscosity and reduction of solute movements. Mishra and Metzger (2019a) also used high temperature (50°C) NF to concentrate MPC80 and achieved the level of solids ~29.5% in the retentate. In the same paper, author also described that the permeate flux indicated longer filtration time even at high temperature NF. The major reason of limited permeate flux through NF might be the concentration polarization because of the terminal flow of feed. Marella et al., (2011) successfully utilized wide pore UF membrane (average pore size 0.01 μm) in plate and frame filtration (PF) system for the whey protein concentration. Nelsen (1977) mentioned that albumin can be concentrated up to 40% TS by using PF system. In PF, flat sheet membranes are packed with screen support (plate) in which the permeate coming through the membrane is collected and removed (McGregor, 1986). Each sheet of membrane in PF packing acts as a new membrane and with the support of plates, it can tolerate high

transmembrane pressure during processing of high solids or viscous feed (Huter and Strube, 2019). The condition creates lower chance of concentration polarization in the PF system compared to spiral wound which helps to recirculate the feed stream at high velocities tangential to the plane of the membrane and increase the mass transfer coefficient (Nelsen, 1977). Increasing TS not only reduces the volume for transportation but also saves the cost of energy during spray drying and improves the functional properties of various dairy ingredients (Mistry, 2002).

Some of the dairy industries has been utilizing PF system to produce whey protein concentrate and whey protein isolate. But the system has been used limitedly to concentrate MPC. Unlike in NF, the tangential flow of feed reduces the possibilities of concentration polarization. However, when the level of solids increased, the viscosity of MPC80 goes up which again can be the hurdle in feed flowing during membrane filtration. According to past studies, the viscosity of milk concentrates containing high proteins can be lowered by increasing the temperature (Mishra and Metzger, 2019a; Morison et al., 2013). High temperature breaks the apparent protein layer formed on the surface of membrane and thereby increased the permeate flux and performance of membrane (Sood and Kosikowski, 1979). Hagen–Poisellue law described that, high temperature also increases the diffusivity of solutes from the membrane surface into the bulk stream and helps in the dispersion of polarized layer. So, the tangential feed flowing, and high temperature processing can improve the filtration performance of PF system. The objective of this study was to evaluate the processing variables to get the highest level of solids of MPC using PF at different temperatures and assess the quality of MPC retentates and powders after drying.

MATERIALS AND METHODS

Experimental Design

Bovine milk (5000 lb) was skimmed and UF in the Davis Dairy Plant at South Dakota State University to manufacture MPC which was further concentrated in PF system at room temperature (22°C) and high temperature (50°C). The high temperature was set by considering the heat sensitivity of whey proteins. Three different PF treatments were conducted such as: PF at 22°C (PF22), PF at 50°C for medium solids (PF50MS), PF at 50°C for high solids (PF50HS). Milk protein concentrate production or concentration variables were evaluated during UF and PF operations. Retentate from UF (feed) was taken as a control to compare the quality of PF retentates. Certain amount of feed as well as retentate from all PF treatments were dried immediately after filtration using spray dryer to get MPC powder.

Ultrafiltration

To produce MPC, pasteurized skim milk was passed through UF membrane made of polyethersulphone material (Parker Hannifin Corp., USA; model SD 6438) with a molecular weight cut-off 10 kDa in a spiral wound set-up. Ultrafiltration was operated between 4 to 9°C with an average total fluid pressure 400 kPa (base: 300 kPa and boost: 100 kPa) through 4 loops having a diameter 0.16 m and length 0.96 m with the spacer 1.125 mm, and surface area 16.7, 16.7, 18, 18 m² respectively for 4 membranes. Diafiltration (DF) was started when the total protein (TP) to total solids (TS) ratio became 0.68 and recycling of retentate was continued until getting the ratio ~0.8 and TS ~20%. Retentate was collected in a crystallizer tank and kept agitating overnight at 4°C. Composition of the skim milk, feed and retentate samples collected during UF/DF

processing were analyzed immediately using FTIR Component Analyzer (Bentley Instruments Inc., Ireland) to regulate the amount of protein and TS.

Plate and Frame Filtration

The MPC80 obtained from UF was further concentrated using a set of flat sheet spiral membranes (total surface area 3.3 m² with a molecular weight cut-off 10 kDa) packed in a pilot scale PF system (Alfa Laval M37). The feed material was preheated to 22- and 50°C for PF22 PF50HS treatments respectively and then filtered in PF system at respective temperatures until the transmembrane pressure difference crossed 9 bar (inlet~11.5 and outlet ~2.5 bar). For the PF50MS treatment, we did not run the separate trial, but we had collected some portion of the retentate and permeate samples from PF50HS treatment when the TS in the retentate was achieved ~30% and the weight of samples taking out were recorded. At the end of each filtration, all the permeate was mixed properly, weighed, and permeate composite samples were collected for further analysis. Some portion of the feed and final retentate was collected and dried immediately in spray drier. About 200 mL of retentate samples from each trial was collected aseptically and stored at 4°C for maximum 72 h to analyze their microbial and physicochemical qualities.

Spray Drying

About 15 lb of feed and final retentates from PF treatments were dried in a spray dryer (Niro dryer Model 1, Niro Inc., Columbia) using an external mix air atomizing nozzle (Spraying Systems Co., model SUE18A). The feed and retentates from PF22 treatment were preheated to 50°C in hot water before spray drying whereas retentates from high temperature PF treatments (PF50MS and PF50HS) were fed directly into the drying

system. The feeding channel was connected to the heat exchanger system (50°C) to maintain the uniform flow of MPC fluid in spraying system. The inlet and outlet temperatures used for the drying were optimized and set at 175°C and 85°C respectively with the air flowrate and pressure 2 scfm and 482 kPa and the fluid flowrate and pressure 7.5 Lh⁻¹ and 240 kPa respectively. Drying was carried out until getting 2 Kg of powder samples for all treatments. Some portion of the powder samples were collected in sterile pouches, sealed immediately and stored at 4°C for microbial analysis and future use. Other samples were stored at room temperature at airtight containers for the quality tests at fresh and stored conditions.

Production/Concentration Variables

Total solids of final retentates and permeate composites from PF treatments were measured instantly using microwave moisture analyzer (CEM Corporation, North Carolina, USA). Permeate flux was recorded in every 10 minutes until the transmembrane pressure difference become 9 bar (inlet~11.5 bar and outlet ~2.5 bar) and the average permeate flux was calculated using equation 1. Volumetric concentration ratio (VCR) was calculated using equation 2. Viscosity of feed and final retentate was measured right after the collection of samples by using a rotational rheometer (MCR 92, Anton Paar Ltd., UK) equipped with bob-and-cup configuration. Viscosity was recorded at 22°C and 50°C at constant shear rate of 100 s⁻¹.

$$\text{Flux} = \frac{\text{Total volume of permeate (L)}}{\text{Effective membrane area (m}^2\text{)} \times \text{time (h)}} \quad [1]$$

$$\text{VCR} = \frac{\text{Initial volume of feed}}{\text{Final volume of retentate}} \quad [2]$$

Compositional Analysis

Feed sample was collected directly from the bulk-tank after 12 h of agitation. Retentate samples were collected before stopping the PF operation whereas the permeate composite samples were made by mixing the whole permeate collected from a batch. All the samples were immediately refrigerated at 4°C. Total nitrogen, non-casein nitrogen (NCN) and non-protein nitrogen (NPN) contents were assessed from Kjeldahl method as mentioned in AOAC (2000a) and for the TP calculation, a factor 6.38 was multiplying with the total nitrogen and expressed as percentage of TS. Amount of lactose in the samples was measured by using a high-performance liquid chromatography (HPLC)-based method as described by Amamcharla and Metzger (2011). Mineral profile was analyzed by using inductively coupled plasma-optical emission spectroscopy as approved by AOAC (2005). Crude fat content was assessed by modified Mojonnier method whereas total ash content by gravimetric method as mentioned in AOAC (2000b).

Protein Fractions

Milk protein fractions of the samples (skim milk, feed, retentates and permeate composites from PF) were analyzed by using capillary electrophoresis system (Beckman P/ACE MDQ, Beckman Coulter, Fullerton, CA, USA) outfitted with a UV detector set at 214 nm. Samples were prepared according to the method described by Salunke et al., 2011. Samples were diluted with HPLC grade water to get $\leq 0.1 \text{ mg mL}^{-1}$. Separation of different proteins was done via a 50 μm wide and 30.2 cm long fused silica capillary. Molecular weight of proteins in the sample were compared with the SDS-MW size standard (recombinant proteins 10 to 225 kDa supplied with the ProteomeLab SDS-MW Analysis Kit) was used to calibrate the gel. 5 μL β -mercaptoethanol was added to each

microfuge vial containing diluted SDS-MW (10 μ L in 85 μ L of sample buffer). Ready vials were heated in a water bath at 90°C for 10 min. Separation was performed at constant voltage of 15 KV, 25°C temperature and 20 bar pressure with reverse polarity in SDS-MW gel buffer for 30 min. A capillary preconditioning was done in six-run cycle. Sample was injected electrokinetically for 20 s at 5 KV. To operate the machine, a 32 Karat™ Software (Beckman Coulter, Inc.) was used. Percentage of protein fractions were analyzed based on the area of peaks of electropherogram. Low molecular weight peptides (LMWP) and high molecular weight peptides (HMWP) were calculated from the cumulative area of peaks before the peak of α -LA and after the peak of κ -CN, respectively.

Microbial Examination

Standard plate counts (SPC) of feed, PF retentates and all MPC powders was enumerated by standard agar method as described by Laird et al. (2004). Feed and retentate samples were measured in g in its place of mL, because the protein gel was solidified after cooling, and diluted 100 times (wt/v) with sterilized phosphate buffer saline PBS. MPC powder was reconstituted by dissolving 11g powder in 99 ml sterilized phosphate buffer saline (PBS) and then mixed in stomacher machine. From each diluted solution, a 100 μ L of sample was mixed aseptically with sterilized standard agar media (~15 g) by pour plate technique in petri-plate and incubated at 37°C for 24 h. Microbial colonies were counted and calculated as colony forming units (CFU) per gram sample and presented in base ten logarithm.

MPC powder was also tested for aerobic mesophilic spores (AMS) using tryptic soy agar (TSA) medium as described by Kent et al., (2016). MPC powder sample was

reconstituted by dissolving 11g powder in 99mL PBS and heated to 80°C for 12 min, cooled to room temperature and diluted 100 times (v/v) with PBS by serial dilution method. A 100μL of diluted sample was dispensed on the solidified TSA media and distributed by streaking method then incubated at 37°C for 48 h. Colonies of spores were counted as CFU per gram sample and presented in base ten logarithm.

Physical Properties of MPC powder

Loose and tapped density of MPC powder was measured as mentioned in IDF Standard 134A:1995. In brief, MPC powder was poured in a dry pre-weighed 100 mL calibrated glass cylinder up to the mark of 100 mL without shaking and weighed to calculate loose density by using equation 3. The cylinder used for the loose density was further tapped for 100 times using Bulk Density Apparatus (UNILAB-009, India) and final volume was measured to calculate tapped density by using equation 4. Flowability of MPC powders was assessed in terms of compressibility index also known for Carr's index (equation 5) by using the values of loose density and tapped density.

$$\text{Loose Bulk Density} = \frac{\text{Weight of powder (Kg)}}{\text{Volume of powder before tapping (m}^3\text{)}} \quad [3]$$

$$\text{Tapped Bulk Density} = \frac{\text{Weight of powder (Kg)}}{\text{Volume of powder after tapping (m}^3\text{)}} \quad [4]$$

$$\text{Carr's index (\%)} = \frac{\text{Tapped density} - \text{Loose density}}{\text{Tapped density}} \times 100 \quad [5]$$

Statistical Analysis

Replicated data were analyzed statistically by using Agricolae: statistical packages for agricultural research in R programming language (version 3.5.2) developed by R core

team. Honest Significant Difference test was used to determine differences between treatment means and the level of significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Production/Concentration Variables

During MPC production using UF/DF, TS and viscosity of feed was measured as production variables whereas average permeate flux, average volumetric concentration ratio (VCR), TS and viscosity of final PF-retentates, and TS of permeate composites were measured as concentration variables during PF (Table 1). Average permeate flux was significantly ($P < 0.05$) increased with increasing temperature and length of filtration. The highest ($11.18 \text{ Lm}^{-2}\text{h}^{-1}$) and lowest ($8.76 \text{ Lm}^{-2}\text{h}^{-1}$) flux was observed for PF50HS and PF22 treatments, respectively. Baker (2004) mentioned that operating conditions such as feed temperature influences both permeate flux and solute rejection in membrane filtration. This is mainly due to the decrease of feed viscosity with an increase of feed temperature. However, permeate flux reduces over time as solids level increased in the feed side. Mishra and Metzger (2019a) observed that the average permeate flux was $3.2 \text{ Lm}^{-2}\text{h}^{-1}$ when MPC80 was processed using NF at 50°C , which was at least 3 times less when compared to PF at 50°C in the current study. Properties of feed including pH, feed viscosity, calcium solubility, protein charge, and solubility also affect permeate flux rates (Rao et al., 1994; Jelen, 1979). According to Kessler et al., (1982), the proportion of calcium associated with the protein micelle determined the extent of concentration polarization during ultrafiltration which is closely related to permeate flux. Similarly, Howell et al. (1981) mentioned that, in ultrafiltration of protein rich solutions, flux declination started by accumulating protein and other charge particles at the membrane

surface due to concentration polarization. In addition, the viscosity of retentate increased and formed a gel on the surface of membrane, which impacted severely in permeation (Howell et al., 1981). The lower permeate flux in the PF22 treatment might be due to the higher viscosity that enhances the concentration polarization at membrane-feed interface. Similar information has been provided by Pompei et al., (1973) and Rash (1976). Breslav and Kicullen (1977) claimed that, flux can be decreased by 3% in each reduction of temperature by 1°C.

Volumetric concentration ratio was also increased with filtration temperature. The highest VCR (1.88) was for the PF50HS treatment however the VCR for the PF22 and PF50MS treatments were statistically similar ($P > 0.05$). For the cheese milk fortification, DMI (2005) suggested to make UF milk of VCR 3 to 5. Unlike filtering skim milk (TS~10%) in UF, here we have concentrated UF milk using PF, the VCR achieved can be low because of the high concentration of feed (TS~20%). According to USEPA (2002), VCR helps to estimate the sensitivity of the method applied in membrane filtration. In suspension mode system, the VCR in feed side of membrane filtration is >1 which can significantly affect the quantity of particulate matter. Except temperature, pressure also can be the factor to push water through the pores of membrane which can influence on the VCR. In our current research, the VCR recorded for the PF system was agreed to the description of USEPA (2002).

The level of TS was increased significantly ($P < 0.05$) in the PF retentates and the highest (34.24%) TS was obtained for the PF50HS treatment whereas the lowest (26.83%) for the PF22 treatment. Increased PF temperature could be the major factor to increase the level of TS in PF50HS treatment. Mishra and Metzger (2019a) studied in

MPC80 concentration using NF at 22°C and 50°C and they observed 25.7 and 29.7% solids, respectively, in the final retentate of NF and they also mentioned that, the difference in solids between low and high temperature NF was lower (4%) when compared to the TS (~7.5%) of PF retentate processed at same temperature. Sood and Kosikowski (1979) also found similar level of TS in the retentate when they processed skim milk at high temperature (60°C) using UF. Similarly, the level of TS in the permeates, range 1.11 to 1.45%, was also higher in the high temperature PF treatments (Table 1) which were significantly ($P < 0.01$) different among the PF treatments. As the PF membrane is a kind of UF membrane, it allows lactose and minerals in the permeate (Berk, 2013). High temperature enhances the removal of small molecular weight solutes from the membrane which can increase the level of solids in the permeate. According to Wijmans and Baker (1995), increase in feed temperature results in a higher permeation of smaller particles such as salts etc., due to a higher diffusion rate in membrane filtration. Some low molecular weight peptides also can pass through the UF membrane (Vandanjon et al., 2007) and may raise the total solids of permeate. Montella (2008) found higher conductivities and TS in the permeates of skim milk processed from UF at 45°C than at 15°C and claimed that temperature of the feed altered the membranes configuration as well as grouping of the molecules. Our results showed that, even at same filtration temperature, longer the filtration time higher the solids drain from the membrane.

Viscosity of the feed and PF retentates were evaluated at 22°C and 50°C and results has expressed in Table 1. Results showed that, even the small increment in TS, the viscosity was raised dramatically. For the PF50HS treatment, the viscosity of final

retentate having TS 34.24% was 12805 cP and 1872 cP for the temperatures 22°C and 50°C, respectively. At low temperatures, molecules of fluid material are tightly bound to each other and they have less mobility cause viscous in nature but when we heat the material, the kinetic energy increased which makes the molecules more mobile and reduces the viscosity (Seeton, 2006). Increasing viscosity reduces the pressure and enhance layer formation on the membrane which impacted on permeate flux, and eventually effects on filtration efficiency (Akoum et al., 2005). In the current study, high temperature PF treatments had higher permeate-flux which could be the cause of lower viscosity of feed at higher temperatures. Higher viscosity of MPC would hampered in the formation of smaller droplets during atomization which might impacts on the powder characteristics after spray drying (Rupp et al., 2018). The viscosity of feed for PF was 79 cP which was reduced to 10 cP when measured at 50°C which improved the filtration efficiency in the PF50MS and PF50HS treatments. Similarly, heating the PF retentates up to 50°C before feeding into spray drier reduced the viscosity which helped in easy going in the spray channel. Overall, high temperature greatly impacted on the processing variables during MPC concentration using PF system.

Compositional Analysis

Composition of retentates. The compositional analysis including TP, total ash, lactose, crude fat, NPN, and NCN of feed and PF retentates was done and the result has expressed in Table 2. Amount of TP was significantly ($P < 0.05$) raised in the retentate of high temperature PF treatments with the range of 81.77 to 88.13%. Increasing the level of TS also contributed to TP increment. High temperature impacted significantly ($P < 0.05$) to increase the TP content in the PF retentates. Results also showed that, lactose and total

ash content were significantly ($P < 0.05$) reduced in the retentates from high temperature PF treatments. Similar levels of lactose and total ash contents were determined by Sood and Kosikowski (1979), and Mistry (2002) when skim milk was ultrafiltered at high temperatures, 60°C and 38°C respectively, to get the final TP content ~80% in the retentate. Crude fat content was found in increasing trend with increasing solids, however there was not a significant ($P > 0.05$) difference between feed and low temperature PF treatment. The highest level of crude fat content (3.43%) was in the retentate of PF50HS treatment. Most of the milk lipid is in the form of a globule with the diameter range 0.1 to 15 μm (Walstra, et al., 2006) which can easily be retained by the UF or PF membrane (pore size 0.01 μm) and increase the concentration. So, the level of crude fat in the final retentate of UF or PF depends on the fat- and fat-soluble substances available in the skim milk which was used as feed for UF. Compositional information of MPC85 powders mentioned by Babu and Amamcharla (2018) and O'Donnell and Butler, (1999) also agreed to the results of our current study.

The NCN, which consists whey proteins and NPN, was decreased significantly ($P < 0.05$) in the high temperature PF retentates. The range of NCN was 1.93 to 2.70% based on TS. The amount of NPN in feed or PF-retentates was very low (0.06-0.09%) and statistically similar ($P > 0.05$). So, majority of NCN in MPC is covered by whey proteins. At higher temperatures and longer filtration time, NCN was in decreasing trend, which reflects that some of the whey protein fractions was either drained in the permeate or formed complex with the casein proteins (Table 2). O'Donnell and Butler, (1999) used a marketed MPC85 in their experiment and determined that there was 2.8% NCN, which was close to the result of current research. Non-protein nitrogen is a group of nitrogenous

substances having nearly 5% of total nitrogen available in bovine milk including urea, low molecular weight peptides, free amino acids, creatine, ammonia etc. (Alichanidis et al., 2016). These substances may vary with season, feeds, herd, breed, and lactation stage (DePeters and Ferguson, 1992). Most of the NPN available in the bovine milk permeates through the UF membrane (Kelly, 2011) causes very low concentration in the UF and PF retentates, which agreed to our results (Table 2). The concentration of NCN and NPN might impacts on the functionality of MPC such as solubility and other special applications (Kelly, 2011). At high temperature processing of milk, NCN can increase due to the denaturation of whey proteins and sometimes casein as well which can further degrade to NPN (Ismail et al., 1969). In our current study, the degraded products formed as NPN might have passed through the UF or PF membrane which did not make any impacts on final retentates of PF.

Though, MPC is produced using UF by removing most of the lactose and minerals and the extent of removal depends on the requirement of protein in the final retentate (O’Kennedy, 2009). As MPC is a potential ingredient in cheese-making, minerals of MPC mainly Ca and P are crucial in the coagulation process and hence on cheese-making efficiency which is best explained by Lucy and Fox (1993). We analyzed feed and PF retentate samples for the major minerals such as Ca, P, Na, K, Mg and S and found in the range of 1999 to 2403, 1308 to 1447, 198 to 245, 421 to 456, 123 to 149, and 26 to 34 mg per 100g retentates on dry weight on the basis of TS (Table 3). Result showed that high temperature PF did not have a significant ($P > 0.05$) impact on the major minerals of MPC retentates and the level of minerals of all types of PF treatments are statistically similar ($P > 0.05$) to the Feed. Fischbach-Greene and Potter (1986) found that the individual mineral

available in skim milk was retained 50 to 90% during ultrafiltration and the retention was not affected by concentration level of retentates, which was agreed to the results of our current research. The level of minerals or salts in the UF retentate depends on the extent of diafiltration (Marella et al., 2015). Ye (2011) determined the calcium and sodium in MPC having 81.5% protein and found 2230 and 70 mg per 100g respectively, where the level of calcium agreed to our findings, but sodium was about 3 times higher in our case. Level of calcium determines the functionality mainly solubility of MPC. Marella et al. (2015) also determined that modification of MPC by injecting carbon dioxide during UF reduced ash (total minerals) by 28% and calcium by 34%, which not only reduces the viscosity of retentate but also improved and maintained the solubility of MPC powder after drying. Other milk minerals, such as P, Zn, and Mg can also be found in the casein micelle whereas S is found in whey protein. So, fortifying variety of supplementary foods with MPC not only add proteins but also includes essential milk minerals.

Composition of permeates. In the current research, the pore size of UF-membrane to produce feed material and the pore size of PF-membrane to concentrate the feed is similar. However, the difference in processing parameters influence in the permeation of solutes during filtration. Permeate composites from three different PF treatments were analyzed for total protein, total ash, lactose, NPN and NCN and the results has shown in Table 4. Total protein was increased significantly ($P < 0.05$) in the permeates of high temperature PF. Total ash and lactose contents in the permeates were decreased with increasing time and temperature of filtration however values were statistically similar ($P > 0.05$) for all PF treatments. There was limited published articles related to the

concentration of MPC by using plate and frame filtration and was hard to find the related information such as composition of retentate or permeate.

High temperature PF did not have a significant ($P > 0.05$) impact on the NPN content of permeates. The level of NPN was in the range 0.49 to 0.67%. From the results, it can be said that the chance of permeating nitrogenous compounds is less compared with lactose and total ash. However, when the filtration elongated, the NPN level was slightly higher in the permeate of PF50HS treatment which might be due to the formation of smaller peptides in the circulating feed by the action of proteases and passed through the PF membrane. The level of NCN was increased significantly ($P < 0.05$) in the permeates of high temperature PF and was in the range 0.95 to 1.56%. Results indicate that some whey proteins can be leaked through the PF-membrane when processed at higher temperatures. Brans et al. (2004) suggested that passing nitrogenous compounds in the permeate is dependent on the membrane properties which can be influenced by feed temperature and processing variables of the typical membrane process (Alkhatim et al., 1998), which was agreed to our findings.

Protein Fractions

Protein Fractions of retentates. Protein fractions of feed and PF retentates from different treatments were compared with the skim-milk and the results has shown in Table 5 and an electrophoretogram of MPC concentrate from one of the PF treatments has been presented in Figure 2. High temperature did not have a significant ($P > 0.05$) impact on the major protein fractions: β -LG, β -CN and α_{s1} -CN compared with the skim milk. The concentration of LMWP in the PF retentates was in the range of 3.25 to 5.13% and was statistically similar ($P > 0.05$) among the PF treatments. Though, peptides have

the antioxidant activity which is assumed to be good for human health, low molecular weight peptides may have the negative impacts on the emulsification capacity of milk protein (Kilara and Panyam, 2003). Formation of peptides due to the heat treatment in milk may alter the sensorial (bitterness) and chemical (Millard browning etc.) properties of the final product (Ritota et al., 2017).

A major whey protein fraction such as β -LG was found in the range 8.60 to 10.37% and did not show any treatment effect ($P > 0.05$). Another whey protein fraction, α -LA, was decreased significantly ($P < 0.05$) in the retentates of high temperature PF treatments. Whey proteins are more heat sensitive than CN and undergo denaturation and form complexes with CN at high temperature processing (Ritota et al., 2017). Researchers claimed that the pores in the UF membranes are large enough to allow some whey proteins to pass in the permeate and the size of pores can also be expanded at higher temperatures (Pompei et al., 1973, Bastian et al., 1991). There was a slight reduction of β -CN (34.03 to 36.05%) in the high temperature PF retentates, however it was statistically similar ($P > 0.05$) to the β -CN fractions of skim-milk. The slight reduction of β -CN at higher temperature PF might have the proteolytic effect and formed the degradation product such as γ -CN by the inherent milk proteolytic enzyme; plasmin (Eigel et al., 1984). The level of γ -CN was significantly ($P < 0.05$) increased with increasing temperature (1.07 to 1.49%). Microbial proteases also can dissociate casein micelles and the activity of the proteases can be increased with increasing microbial load (Ismail and Nielsen, 2010), which was possible at higher temperature PF treatments.

Another major CN fraction in the retentates such as α_{s1} -CN, ranged 35.75 to 38.46%, was highest for the PF22 treatment, however for the high temperature PF treatments the

fraction was similar ($P > 0.05$) to the feed and skim milk. α_{s1} - and α_{s2} -CN also can undergo hydrolysis by the plasmin during processing and storage (Grufferty and Fox, 1988). Another minor CN fraction κ -CN was ranged from 2.91 to 3.86%, which is considered sensitive to the heat treatment however, in the current research high temperature did not make significant ($P > 0.05$) impact on it. At high temperature processing, whey proteins can bind with κ -CN at the surface of the casein micelles to form a complex and the extent of complexing increases with time and temperature (Ismail and Nielsen, 2010). The low level of κ -CN in the feed (2.04%) might be due to hydrolysis by the proteases of psychrotrophs as the feed was produced and stored at refrigeration temperature which leads to form peptides and has best explained by Cromie (1992). There was a small amount ($< 1\%$) of HMWP detected in the feed and retentate of PF22 treatment however the level was lower than that of retentates from high temperature PF treatments. Mostly, HMWP derived from milk are casecidins and isracidin which are the proteolytic derivatives of α_{s1} -CN due to the action of microbial proteases (Hill et al., 1974). Most of the HMWP can further hydrolyze to smaller peptides which can pass with permeates during high temperature PF treatments.

Protein Fractions of permeates. Protein fractions of PF permeates were evaluated and results has shown in Table 6, and an electrophoretogram from electrophoresis analysis has been presented in Figure 3. High temperature PF permeates have significantly ($P < 0.05$) higher amount of LMWP which was increased with increasing filtration time. Some of the proteins undergo proteolysis due to the action of proteases during processing and the reaction rate might increases at higher temperature PF and increase the level of LMWP, which mostly passed through the PF-membranes. We did not

see any CN fractions in the PF permeates. Giori et al. (1985) found that CN undergo slight proteolysis with some of the mesophilic bacteria at between 45 to 50°C. According to Akerstedt et al. (2012), major whey proteins such as α -LA and β -LG undergo proteolysis by mesophilic bacteria, which can be correlated to the slight reduction of α -LA fraction in the retentates from high temperature PF. The concentration of α -LA was nearly four times higher compared to β -LG which might be due to lower molecular weight of α -LA (Aich et al., 2015).

Microbial Examination

The number of microbes in MPC can be increased with multiple handling such as transferring, membrane filtration, holding etc. during MPC production/concentration which might impairs the quality of final retentate. However, the extent of microbial load depends on the processing temperature and length of processing. In the current study we had concentrated MPC at room temperature as well as at 50°C. Both temperatures are suitable for the growth of variety of microorganisms. We had examined standard plate counts (SPC) for the feed and PF retentates, and the result has shown in Figure 1. Counts were significantly ($P < 0.05$) higher in the retentates of high temperature PF which were increased significantly ($P < 0.05$) in the retentates of high temperature PF ($\sim 5 \text{ Log}_{10}\text{CFUg}^{-1}$), however the SPC was comparable to the regulatory standards allocated for fluid milk in USA (Murphy, 2000). Mishra and Metzger (2019a) also found similar results when the MPC was concentrated using NF at 50°C. The SPC in the feed and PF22 treatments were between 3.5- and 4.4 $\text{Log}_{10}\text{CFUg}^{-1}$. High microbial load may act on lactose and proteins, resulting the reduction of pH and develops off-flavor; however, we had dried the final retentate immediately after PF using spray drier. Most of the vegetative cells are

destroyed due to hot air during spray drying and these cells rarely grow further due to low water activity in the powder. MPC powders were also tested for the SPC and result has been compared with the SPC of feed and PF-retentates (Figure 1). Though the SPC was high (4.5- to 4.6 Log₁₀CFUg⁻¹) in the powder from high temperature PF treatments, we did not see any treatment effect ($P > 0.05$) on the SPC of powders. The level of SPC in the powders were under the acceptable limit as described by USDA (2001) for non-fat dry milk powders and comparable to the results obtained by Buehner et al., (2015).

We also enumerated aerobic mesophilic spores (AMS) in the MPC powders and results showed that high temperature did not have a significant ($P > 0.05$) impact on the AMS among the PF treatments. Counts of AMS in the powders from all treatments were under the tolerable limit (< 3 Log₁₀CFUg⁻¹) as defined by US Dairy Export Council. Mishra and Metzger (2019b) also detected similar level of AMS in MPC80 powders obtained from NF at 22- and 50°C. Mistry (2013) mentioned that the load of microbes and spores in the feed or skim milk should be very low to produce high quality MPC. However, except the raw material, there are number of sources for the cross-contamination including filter-membrane, utensils, improper handlings, etc. and the microbes can multiply during subsequent processing. Equipment surfaces used in milk handling are commonly contaminated by microorganisms even after cleaning and disinfection (Marouani-Gadri et al., 2010). Filter membranes, rubber seals, and stainless-steel surfaces in the food industry plants also bear biofilms containing spores and bacteria (Kumar and Anand, 1998), which can be the potential source of contamination of finished products that reduce shelf life or assist in spreading diseases (Brooks and Flint, 2008). Most of the mesophilic spore formers are from *Bacillus* spp. and *Brevibacillus*

spp. and can grow between 30 to 40°C (Willey et al., 2008). Eijlander et al. (2019) mentioned that spores of several strains of *Bacillus* spp. such as *B. subtilis*, *B. thermoamylovoran*, *B. licheniformis* and *B. sporothermmodurans* were the mostly detected mesophiles in the milk powder. In the current study, number of SPC as well as AMS detected in the MPC powders were under the acceptable limit as mentioned in the US dairy standards (USDA, 2001).

Physical Properties of MPC Powder

Physical properties such as moisture content, loose density, tapped density and flowability of MPC powder were assessed and expressed in Table 7. Moisture content of the MPC powders was in the range of 2.04 to 3.51%. Powders from high temperature PF had significantly ($P < 0.05$) lower moisture contents compared to the powders from feed and low temperature PF. However, the level of moisture in the powders from all the treatments was under the acceptable limit ($\leq 5\%$) as mentioned in USDA standards for the spray dried non-fat dry milk (USDA, 2001) which is supposed to be safe for any food application or to store for future use. The moisture content in the spray dried powder depends on drying conditions such as the level of solids, viscosity, flowrate of feed and outlet temperature of hot air. Ziaee (2019) mentioned that, higher TS in feed gives lower moisture product after spray drying because of having smaller proportion of water to be evaporated in same environment compared to lower TS in the feed (Ziaee, 2019). Hence, there was a high chance of getting low moisture product for the high temperature PF treatments, which was agreed to our findings.

Loose density of MPC powders was in the range of 279-300 Kgm⁻³, which was significantly ($P < 0.05$) reduced for the MPC powders from high temperature PF

treatments whereas tapped density, (346 to 369 Kgm⁻³) was statistically similar ($P > 0.05$) for all PF treatments. Mishra and Metzger (2019a) studied on MPC80 concentrated by using NF at 22 and 50°C and observed slightly higher loose density; 353 and 332 Kgm⁻³ and tapped density; 454 and 428 Kgm⁻³, respectively. Jean-Marie et al. (2013) prepared the MPC85 powders prepared by evaporation and spray drying of MPC from UF and tested for the loose and tapped densities and found 356 and 519 Kgm⁻³, respectively. The lower loose and tapped density in the MPC powders from current studies might be due to lower lactose or higher protein contents. Bulk density of MPC powders can also be influenced by the particle size or surface area of the particles and occluded air (Li et al., 2018; Jean-Marie et al., 2013; Sharma et. al., 2012). Similarly, viscous feed for spray drying from same level of solids gives the powder having larger particles because of the larger droplet formation during spraying however the particle size might not be uniform (Santos et al., 2017). Results of current study and similar past studies showed that, increasing protein content in spraying feed and pretreatments prior to drying impacted on the bulk density of MPC powders after drying.

All MPC powders had narrow range of flowability in terms of Carr's index (18.59 to 19.34) which explained that MPC powders from all treatments were of fair flowing category. Flowability of the powders from all treatments were statistically similar ($P > 0.05$). According to Sharma et al. (2012), MPC powders having protein content $> 80\%$ have poor flowability. Silva and O'Mahony (2017) reported that the skim milk powder and MPC70 powder was categorized as free flowing and easy flowing powders, respectively. The free or easy flowing might be due to more availability of lactose in those powders. With increasing protein content, the angle of internal friction increased

which increased cohesiveness and finally impacted on the flowability of the powder. In contrary, the inter-particle interactions decreased with increasing particle size and hence improves flowability (Fitzpatrick, 2004). MPC powders in our research had higher protein (81 to 88%) and less lactose (4.1 to 7.5%) contents so, the flowability might be poorer compared to low protein high lactose containing MPC powders. Fitzpatrick (2007) mentioned that, increased moisture reduced the flowability of powders by bridging liquids and capillary interactions within the particles, so the flowability of MPC powder can be poorer with increasing storage time.

CONCLUSIONS

So far, PF is not a common practice in dairy industry to produce MPC. We studied the production variables to concentrate MPC80 in pilot scale PF system at 22- and 50°C. High temperature PF impacted significantly to increase average permeate flux, VCR, level of TS in retentate (~14% more compared to feed TS) and TP to TS ratio (~88%) however, the removal of solids and NPN was also higher in the permeates of high temperature PF treatments. High temperature PF impacted to increase γ -CN and decrease α -LA which might be the result of increased proteolytic activity at elevated temperature. The level of SPC and aerobic mesophilic spores in MPC powder after drying was within the acceptable limit as mentioned in the USDA standards. The tapped density and flowability of the MPC powder did not show any differences among the PF treatments. From this study, it can be claimed that PF could be a potential alternative to concentrate MPC with increasing TP at a time. Hence, high temperature PF helps in saving large amount of energy in filtration as well as in spray drying of MPC by retaining most of the physicochemical qualities of MPC powders.

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Table 1. MPC processing variables (mean, n=3)

Treatments ¹	Flux ² (Lm ⁻² hr ⁻¹)	VCR ³	Viscosity ⁴ at 22°C (cP)	Viscosity ⁴ at 50°C (cP)	Total Solids of Retentate (% wt/wt)	Total Solids of Permeate (% wt/wt)
Feed	-	-	79 ^c	10 ^c	20.23 ^d	-
PF22	8.76 ^C	1.51 ^b	577 ^c	279 ^b	26.83 ^c	1.11 ^c
PF50MS	10.50 ^b	1.60 ^b	1513 ^b	324 ^b	29.92 ^b	1.22 ^b
PF50HS	11.18 ^a	1.88 ^a	12805 ^a	1872 ^a	34.24 ^a	1.45 ^a

^{a-d}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹Treatments: Feed was retentate from ultrafiltration of skim milk; PF22 was the retentate from plate and frame filtration of feed at 22°C; and PF50MS and PF50HS were the retentates from the plate and frame filtration of feed at 50°C for medium and high solids, respectively.

²Permeate flux during plate and frame filtration

³Volumetric concentration ratio during plate and frame filtration

⁴Viscosity of feed or final retentates from plate and frame filtration

Table 2. Mean (n=3) compositional analysis of retentates (% dry weight basis)

Treatments ¹	Total Prot	Total Ash	Lactose	Crude Fat	NPN ²	NCN ²
Feed	81.77 ^b	7.71 ^a	7.52 ^a	2.72 ^c	0.09 ^a	2.70 ^a
PF22	83.04 ^b	7.12 ^b	7.00 ^a	2.92 ^c	0.07 ^a	2.51 ^{ab}
PF50MS	87.37 ^a	4.97 ^c	4.37 ^b	3.16 ^b	0.07 ^a	2.24 ^{bc}
PF50HS	88.13 ^a	4.91 ^c	4.08 ^b	3.43 ^a	0.06 ^a	1.93 ^c

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹Treatments: Feed was retentate from ultrafiltration of skim milk; PF22 was the retentate from plate and frame filtration of feed at 22°C; and PF50MS and PF50HS were the retentates from the plate and frame filtration of feed at 50°C for medium and high solids, respectively.

²NPN=non-protein nitrogen; NCN=non-casein nitrogen

Table 3. Mineral analysis (mg per 100g dry solids, mean, n=3) of PF retentates

Treatments ¹	Ca	P	Na	K	Mg	S
Feed	1999 ^a	1308 ^a	231 ^a	423 ^a	149 ^a	29 ^a
PF22	2307 ^a	1447 ^a	214 ^a	430 ^a	123 ^a	30 ^a
PF50MS	2162 ^a	1311 ^a	245 ^a	456 ^a	139 ^a	34 ^a
PF50HS	2403 ^a	1384 ^a	198 ^a	421 ^a	139 ^a	26 ^a

^aValues with the same superscript within a column are not significantly different ($P > 0.05$).

¹Feed is the retentate from ultrafiltration of skim milk; PF22 is the retentate from plate and frame filtration of feed at 22°C; and PF50MS and PF50HS are the retentates from the plate and frame filtration of feed at 50°C for medium and high solids, respectively.

Table 4. Permeate composition (% dry basis, mean, n=3)

Treatments ¹	Total Protein	Total Ash	Lactose	NPN ²	NCN ²
PF22	9.54 ^b	16.75 ^a	73.72 ^a	0.56 ^a	0.95 ^b
PF50MS	12.28 ^{ab}	15.30 ^a	73.52 ^a	0.49 ^a	1.45 ^a
PF50HS	14.95 ^a	14.73 ^a	71.00 ^a	0.67 ^a	1.56 ^a

^{a-b}Values with the same superscript within a column are not significantly different (P >0.05).

¹PF22 is the permeate from plate and frame filtration of feed at 22°C; PF50MS and PF50HS are the permeates from plate and frame filtration of feed at 50°C for medium and high solids, respectively.

²NPN=Non-protein nitrogen, NCN=Non-casein nitrogen.

Table 5. Protein fractions (% of total protein; mean, n=3) of skim-milk and retentates

Proteins ³	Skim-milk ¹	Feed ²	PF22 ²	PF50MS ²	PF50HS ²
LMWP	4.86 ^a	3.46 ^a	3.25 ^a	5.13 ^a	3.94 ^a
α -LA	3.00 ^a	2.19 ^{bc}	2.64 ^{ab}	1.91 ^c	1.86 ^c
β -LG	9.76 ^a	9.75 ^a	10.37 ^a	8.60 ^a	9.33 ^a
γ -CN	0.19 ^c	0.39 ^c	0.42 ^c	1.07 ^b	1.49 ^a
β -CN	36.34 ^{ab}	37.37 ^a	36.05 ^{ab}	34.60 ^b	34.03 ^b
α_{s1} -CN	35.75 ^b	36.64 ^{ab}	38.46 ^a	36.41 ^b	35.75 ^b
α_{s2} -CN	6.31 ^{ab}	7.23 ^{ab}	5.65 ^b	8.48 ^{ab}	9.75 ^a
κ -CN	3.56 ^a	2.04 ^b	2.91 ^{ab}	3.86 ^a	3.47 ^{ab}
HMWP	0.00 ^a	0.94 ^a	0.67 ^a	0.00 ^a	0.31 ^a

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹Skim-milk was the source material for feed and retentates of respective treatments.

²Feed was retentate from ultrafiltration of skim milk; PF22 was the retentate from plate and frame filtration of feed at 22°C; and PF50MS and PF50HS were the retentates from the plate and frame filtration of feed at 50°C for medium and high solids, respectively.

³Proteins=protein fractions: LMWP=low molecular weight peptides; α -LA=alpha lactalbumin; β -LG= beta lactoglobulin; γ -CN=gamma casein; β -CN=beta casein; α_{s1} -CN=alpha casein-1; α_{s2} -CN =alpha casin-2; κ -CN=kappa casein; HMWP=high molecular weight peptides.

Table 6. Protein fractions (% of total protein; mean, n=3) of permeates

Treatments ¹	LMWP ²	α -LA ²	β -LG ²
PF22	33.47 ^b	54.69 ^a	11.84 ^a
PF50MS	53.88 ^a	36.13 ^b	9.99 ^b
PF50HS	55.93 ^a	35.50 ^b	8.57 ^c

^{a-c}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹PF22 is the permeate from plate and frame filtration of feed at 22°C; and PF50MS and PF50HS are the permeates from the plate and frame filtration of feed at 50°C for medium and high solids, respectively.

²LMWP=low molecular weight peptides, α -LA=alpha lactalbumin, β -LG= beta lactoglobulin.

Table 7. Physical analysis (mean, n=3) of MPC powder¹

Properties	Feed ²	PF22 ²	PF50MS ²	PF50HS ²
Moisture	3.51 ^a	3.41 ^a	2.32 ^b	2.04 ^b
Loose Density (Kgm ⁻³)	300 ^a	292 ^{ab}	286 ^{bc}	279 ^c
Tapped Density (Kgm ⁻³)	369 ^a	359 ^{ab}	352 ^{ab}	346 ^b
Flowability/Carr's Index	18.59 ^a	18.72 ^a	18.73 ^a	19.34 ^a

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹MPC powder was prepared by spray drying of retentates from different PF treatments

²Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively.

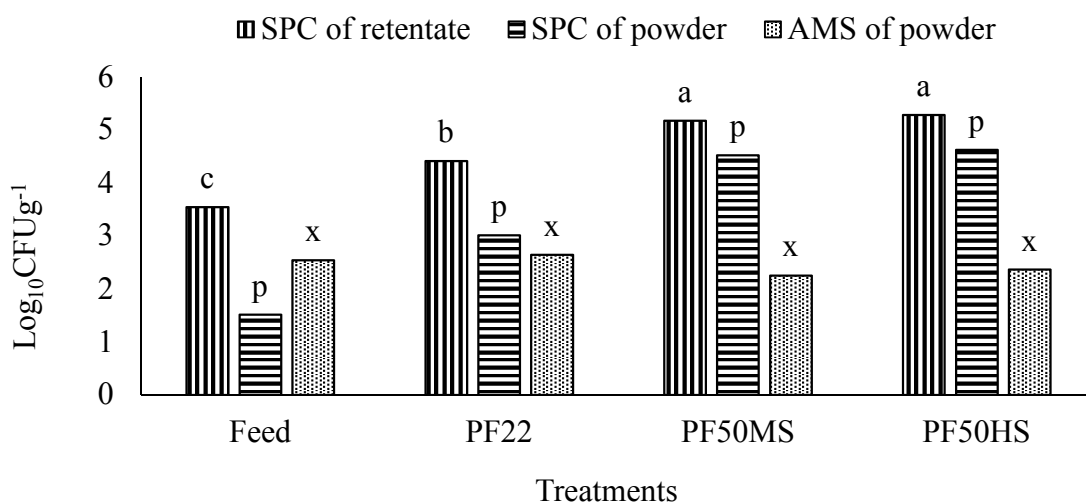


Figure 1. Standard Plate Count (SPC) and Aerobic Mesophilic Spore (AMS) counts in retentate and powders (% mean, n=3) of MPC. For the SPC of retentates, feed is the retentate from ultrafiltration of skim milk; PF22 is the retentate from plate and frame filtration of feed at 22°C; and PF50MS and PF50HS are the retentates from the plate and frame filtration of feed at 50°C for medium and high solids, respectively. For the SPC and AMS of powders, Feed, PF22, PF50MS and PF50HS are the spray dried MPC powders of respective retentates. Values with the same letters (a-c for SPC of retentates, p for SPC of powder, and x for AMS of powder on the bars) are not significantly different ($P > 0.05$) across all treatments.

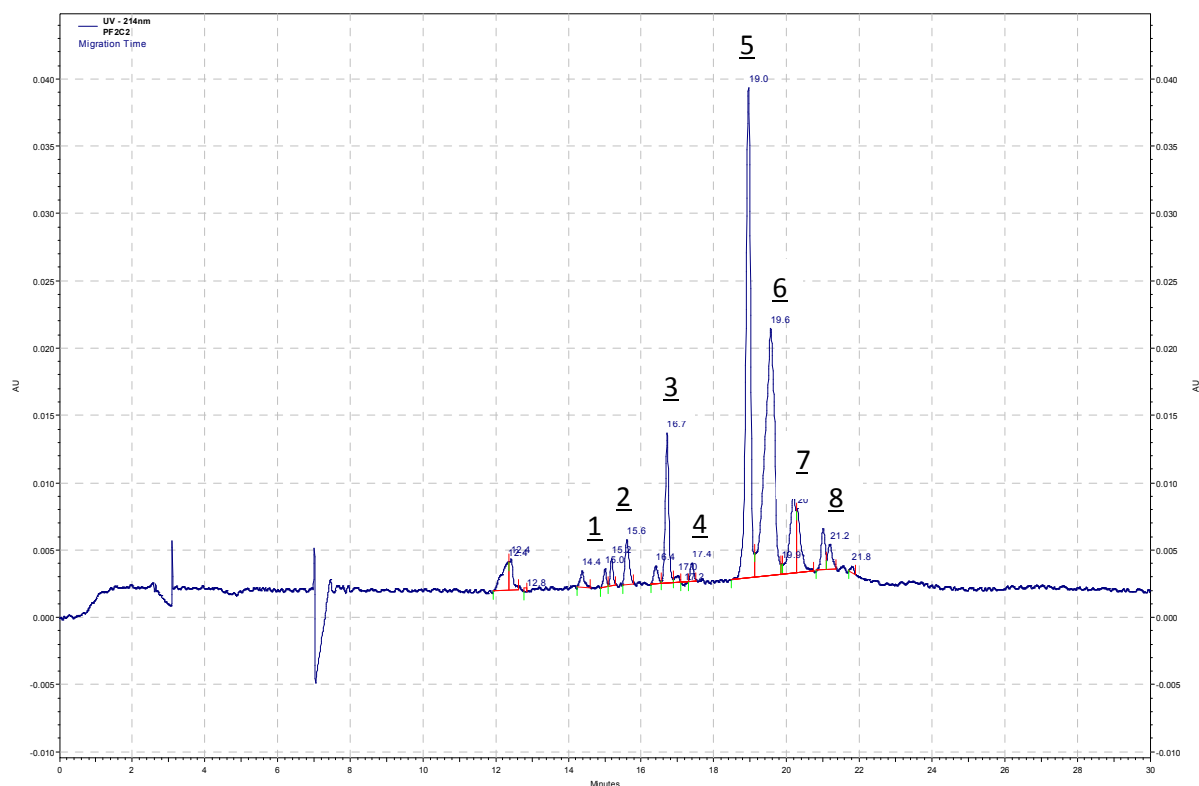


Figure 2. The example capillary gel electrophoresis (CE) electrophoretogram of MPC concentrated from one of the PF treatments. 1=low molecular weight peptides (LMWP); 2= α -lactalbumin (α -LA); 3= β -lactoglobulin (β -LG); 4= γ -casein (γ -CN), 5= β -casein (β -CN); 6= α_{S1} -casein (α_{S1} -CN); 7= α_{S2} -casein (α_{S2} -CN); 8 = κ -casein (κ -CN).

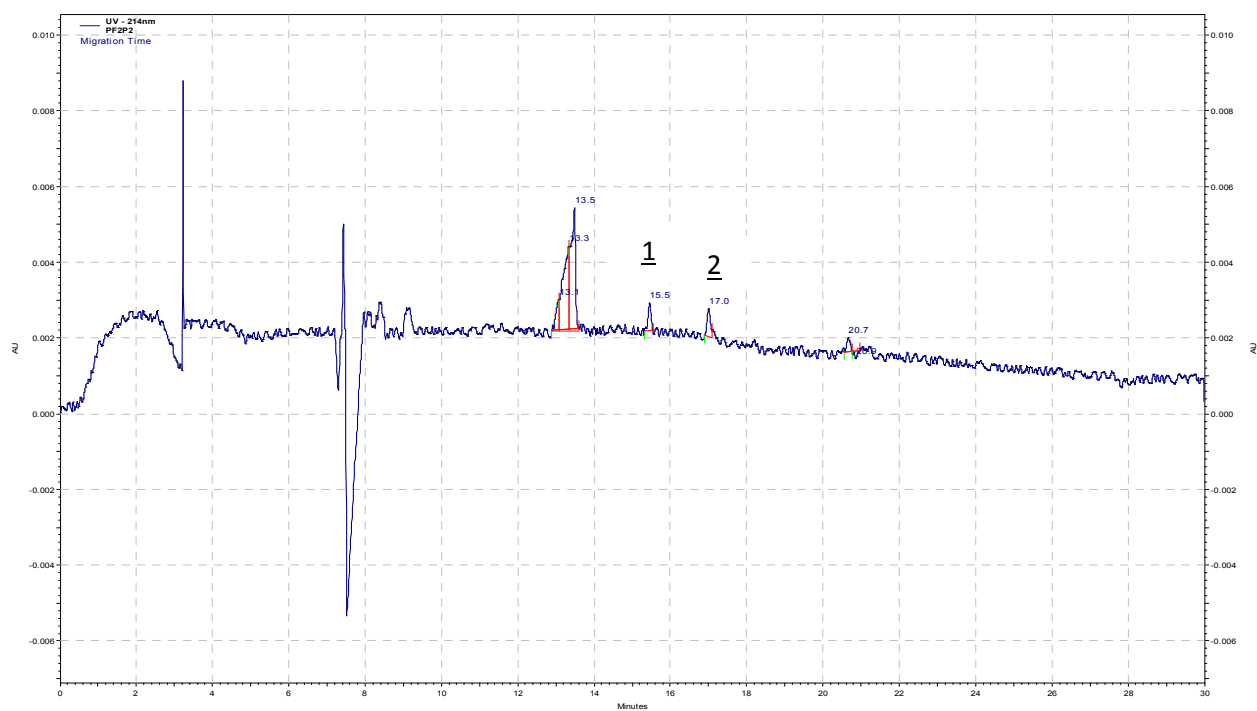


Figure 3. The example capillary gel electrophoresis (CE) electrophoretogram of MPC permeate from one of the PF treatments. 1= α -lactalbumin (α -LA); 2= β -lactoglobulin (β -LG).

CHAPTER 5

Effect of Plate and Frame Filtration Temperature on the Functionality of Milk Protein Concentrate

INTRODUCTION

Milk Protein Concentrate (**MPC**) shows a wide range of functionality in food products development because of its high protein, ranging from 42 to 85% (Patel et al., 2014). In general, skim milk is ultrafiltered to produce liquid MPC and further spray dried to get MPC powder. Nanofiltration (**NF**) is a common practice to increase the level of solids in MPC which can save the cost of energy by lessening water per unit feed material in drying. When NF progresses, viscosity rises with increasing solids results delay in feed flow and the level of concentration polarization on membrane surface also increases (Bacchin et al., 2006). In the associated research we had explored the application of plate and frame filtration (**PF**) at different temperatures to find the better alternative of NF in high solids MPC production. Usually, PF utilized in concentration of fluid materials by pervaporation, reverse osmosis, and ultrafiltration (**UF**) in excessive fouling condition. When viscosity of feed increases during the concentration of protein rich fluids, UF in PF module often used in the final concentration step (Cui and Muralidhara, 2010). Viscosity and final solute concentration in feed impacts on the functionality of dairy powder after spray drying (Wu et al., 2014). Similarly, high temperature processing may result in structural changes in milk proteins by denaturation and complex forming between caseins and whey protein (Fang et al., 2012). Drying temperature and subsequent storage conditions increases the protein interactions which impacts on the functionality of MPC powders (Anema et al., 2006).

Major functional properties of MPC powders are water binding, emulsification, foaming, and heat stability of food items and play important functional role when utilized in yogurt, ice cream, cheese and beverage formulation (Patel et al., 2014). The rate of water absorption can be predicted from dissolution index, wetting time, and solubility (Kneifel and Seiler, 1993). The dissolution behavior of MPC powders are related to their protein content and storage conditions and can be predicted by focused beam reflectance measurement (**FBRM**) method in terms of relative dissolution index (**RDI**) (Babu and Amamcharla, 2018). Solubility of MPC powder depends on the protein content, drying and storage temperature and length of storage (Anema et al., 2006; Gazi & Huppertz, 2015) and can be enhanced by higher rehydration temperature (Udabage et al., 2012). Wetting time of MPC powder rely on the surface composition and density of powder particles, and the temperature of solvent used for wetting (Fitzpatrick et al., 2017). Structure of protein emulsion affects by homogenization condition. In MPC, milk proteins are in aggregated form, so the emulsifying capacity can be poorer than the individual milk proteins (Singh and Ye, 2020). Foamability of MPC depends on the availability of heat-induced aggregation of whey and casein (**CN**) proteins and mixing temperature (Huppertz, 2010; Kamath et al., 2008). The rheological properties of dairy powders depend on protein contents, particle deformation and interaction during rehydration (Vélez-Ruiz et al., 1997, Tasker et al., 2018). In dairy beverage processing, proteins should remain uncoagulated during commercial sterilization. In coagulation, whey proteins interact with caseins through hydrophobic and disulfide bonds (Fox, 1981). Use of MPC in cheese and cheese spread production is increasing (Patel et al., 2014). In cheese application, MPC must have acceptable rennet coagulation time (**RCT**)

(Rehman et al., 2003), which is highly depends on the availability of ionic calcium having role for joining casein micelles together for the coagulation. The decreased ionic calcium during UF can be retained by adding ionic calcium (Robinson and Wilbey, 1998) to improve the rennetability of MPC. The objective of this study was to assess the impact of high temperature PF on the functionality of MPC powders.

MATERIALS AND METHODS

Experimental Design

MPC as feed-material containing ~20% total solids (TS) and ~80% protein based on TS was obtained from ultrafiltration (polyethersulphone, Parker Hannifin Corp., USA; model SD 6438, with a molecular weight cut-off 10 kDa in a spiral wound set-up) of skim milk. Feed was preheated and then concentrated by using PF system (polysulphone, Alfa Laval Module M37, total surface area 3.3 m² with a molecular weight cut-off 10 kDa in a flat sheet module) for three times. Three different PF treatments such as PF at 22°C (PF22), PF at 50°C for medium solids (PF50MS), and PF at 50°C for high solids (PF50HS) were performed. For PF22 and PF50HS treatments, filtration was continued until the transmembrane pressure difference turn into 900 kPa while for PF50MS treatment, retentate was drawn as soon as the level of TS achieved ~30%. Feed and PF retentates were spray dried in a pilot-scale dryer (Niro dryer Model 1, Niro Inc., Columbia) having an external mix air atomizing nozzle (Spraying Systems Co., model SUE18A) system to make MPC powder. Powder samples were tested immediately for their functional properties. Part of the powder sample was stored at 4°C in airtight container for 6 months.

Physicochemical Properties

Heat Coagulation Time. The time required for the visible coagulation when a liquid MPC exposed at high temperature was assumed as HCT and the method was conducted as described by IDF, 1995 with some modification. Reconstituted MPC (10% wt/wt, pH ~6.8) was prepared in a deionized water of 50°C and agitated continuously for 30 minutes using magnetic stirrer (700 rpm) in water bath maintaining temperature 50°C. Three gram reconstituted MPC was taken in a transparent glass vile (8 mL autoclavable E-C vial, Wheaton) and capped air-tight with a heat resistant screw cap. Vials were clamped on the arm of rocker fitted in oil-bath (NSW-199, Narang Scientific Works Pvt. Ltd., India) where a custom-made circulation device was adjusted. Food grade mineral oil was used in the oil bath and the temperature was adjusted at 120°C. The time in min required for the first visible coagulation of reconstituted MPC in the vial was recorded as the heat coagulation time.

Wetting time. The wetting time of MPC powder was determined at specified temperature as mentioned in the method described by IDF (1979) with some modification. Five gram MPC powder was poured with the help of a funnel (made of anti-static material, height 100 mm, lower diameter 40 mm, upper diameter 90 mm) on the surface of deionized water in beaker (diameter 71 mm, height 115 mm). A sieve was used between funnel and beaker for making the uniform fall all over the surface of water in the beaker. The wetting time was recorded at two different temperatures: 22- and 50°C. The time in min required for the complete wetting of MPC powder was noted.

Flowability. The flowability of MPC powder was estimated with the help Carr's index (equation 1) using the values of loose and tapped density of MPC powders (Table 1).

According to Carr (1965), when the Carr's index values of the powder lies between 0 to 11, 11 to 15, 16 to 20, 21 to 25, 26 to 31 and 32 to 37, the flowability of the powder will be categorized as excellent, good, fair, passable, poor and very poor, respectively.

$$\text{Carr's index (\%)} = \frac{\text{Tapped density} - \text{Loose density}}{\text{Tapped density}} \times 100 \quad [1]$$

Relative Dissolution Index

Dissolution property of MPC powders in terms of RDI was evaluated using FBRM method as described by Hauser and Amamcharla (2016). MPC powder was dissolved in deionized water maintained at 25°C to make protein concentration 5% (wt/wt) in the solution. A 250 ml glass beaker equipped with an overhead stirrer having 4-blade impeller (Caframo, Georgian Bluffs, Ontario, Canada) was utilized to dissolve the powder particles at the speed of 400 rpm. An iC FBRM software (version 4.3.391, Mettler-Toledo AutoChem Inc., Columbia, MD) was used for obtaining and checking the FBRM data which could track the number of fine particles having the chord length <10 µm. The dissolution characteristics of MPC powders were monitored in terms of particle counts observed for 30 min. When the powder started dissolution, the counts of fine particles were increased with time which were plotted against powder dissolution time. Area under the fine particle counts curve was calculated by using the trapezoidal rule to describe the powder dissolution characteristics. RDI (%) of MPC powder was determined by using equation [2].

$$\text{RDI (\%)} = \frac{\text{Area under the curve for the PF treatment}}{\text{Area under the curve for the feed}} \times 100 \quad [2]$$

Rheological Properties

MPC powder was reconstituted (10% wt/wt) by mixing in deionized water and stirred for 30 minutes at 22°C. After holding a min, 60 mL of reconstituted MPC was poured into the cup of rheometer (MCR 92, Anton Paar Ltd., UK) with bob-and-cup configuration and then flow behavior was analyzed at shear rate of 1 to 1000 s⁻¹ at 22°C. The graph of shear stress against shear rate was best fitted with Herschel-Bulkley model as mentioned in equation 3.

$$\bar{\sigma} = \bar{\sigma}_0 + K(\dot{\gamma})^n \quad [3]$$

Where, $\bar{\sigma}$ is the shear stress (Pa), $\bar{\sigma}_0$ is the yield stress (Pa), K is the consistency index (Pa.sⁿ), $\dot{\gamma}$ is the shear rate (s⁻¹), and n is the flow behavior index.

Solubility

Solubility of MPC powder was determined as the method described by Haque et al. (2012) with minor modification. MPC was reconstituted with deionized water to make the final concentration 5% (wt/wt) and mixed for 30 minutes using a magnetic stirrer (700 rpm) at 22°C. Forty milliliter of reconstituted MPC was poured into a 50 mL vial and centrifuged at 700xg for 10 minutes. The supernatant was transferred carefully in a dry pre-weighed aluminum bowl and dried overnight at 103±2°C, cooled and weighed. The solubility of MPC powder was calculated using equation 4. To determine the solubility of MPC powder at 50°C, the reconstitution and mixing steps were carried out at 50°C and rest of the steps were followed same.

$$\text{Solubility (\%)} = \frac{\text{dry matter in supernatant}}{\text{dry matter in bulk solution}} \times 100 \quad [4]$$

Emulsifying Capacity and Emulsion Stability

For determining emulsifying capacity, MPC powder was mixed in deionized water and stirred using magnetic stirrer (700rpm) for 60 min at 22°C to make 1% dispersion (wt/wt) and the pH was adjusted to neutral (6.8 to 7.0) by using NaOH. Seven-gram of the dispersion was mixed with 3 g soybean oil in a 50 mL centrifuge tube and heated to 55°C. The hot mixture was homogenized for 60 s at 10,000 rpm using a benchtop homogenizer (Polytron, PT 2500E) to make emulsion. Eight-gram emulsion was transferred to another 15 mL centrifuge tube and then centrifuged at 1100xg for 5 min. The height of the emulsified layer was recorded, and the emulsifying capacity was determined using equation 5.

$$\text{Emulsifying capacity (\%)} = \frac{\text{Height of emulsified layer (mL)}}{\text{Height of total content in the tube (mL)}} \times 100 \quad [5]$$

To determine the stability of the emulsion, the tubes with emulsion were put in water bath at 80°C for 30 min and cooled to room temperature (22°C) and recentrifuged at 1100xg for 5 min. The height of the existed emulsion was recorded, and the emulsion stability was determined using equation 6.

$$\text{Emulsion stability (\%)} = \frac{\text{Height of emulsified layer after heating (mL)}}{\text{Height of total content in the tube (mL)}} \times 100 \quad [6]$$

Whey Protein and Denatured Protein

Whey protein nitrogen was determined from the difference of NCN and NPN (Dupont et al., 2011). The values of NCN and NPN have shown in Table 1. Whey protein was calculated using equation 7 and expressed in the percentage of true protein. When MPC is processed at high temperature, some portion of whey protein undergo denaturation.

The severity of denaturation depends on temperature and length of heating (Wijayanti et al., 2014).

$$\text{Whey Protein (\%)} = 6.38 (\text{NCN} - \text{NPN}) \quad [7]$$

The level of denatured protein, formed due to the number of heat application during MPC production process, was estimated by Kjeldahl method. Test sample was prepared as described by Morr (1985). In brief, 5 g of MPC powder sample was mixed with 60 mL deionized water in a 150 mL conical flask and stirred for 10 minutes at 22°C using magnetic stirrer at 700 rpm. The pH of MPC solution was adjusted to between 4.55 to 4.65 with 0.1M HCl and stirred continuously for next 30 min and the final volume was maintained 100 mL with deionized water which was ready as test solution for the estimation of total nitrogen. Similarly, a 45 mL test solution in 50 mL vial was centrifuged at 3000 rpm for 20 min then the supernatant was filtered using whatman#1 filter paper and the filtrate was used as a test solution for soluble nitrogen. Total nitrogen and soluble nitrogen were determined by Kjeldahl method and the denatured protein was calculated using equation 8.

$$\text{Denatured protein (\%)} = 100 - \left(\frac{\text{Soluble Nitrogen}}{\text{Total Nitrogen}} \times 100 \right) \quad [8]$$

Foaming Capacity and Foam Stability

Foaming capacity of MPC powder was estimated as the method described by Shilpashree et al., (2015). Three-gram MPC powder was whipped vigorously with 100 mL phosphate buffer (0.05 molL⁻¹, pH 7) in an auto-mixer (Osterizer blender, Model 6630) for 6 min at the speed of 11,000 rpm at room temperature. The foam was

transferred instantly in to a 250 mL measuring cylinder quantitatively and the total volume was recorded. Foaming capacity was evaluated by using equation 9.

$$\text{Foaming capacity (\%)} = \frac{\text{Foam volume after whipping} - \text{Liquid volume before whipping}}{\text{Liquid volume before whipping}} \times 100$$

[9]

The measuring cylinder containing foam was kept for 30 min at 22°C without any vibration and the final volume of foam was documented. The foam stability was evaluated by using equation 10.

$$\text{Foaming capacity (\%)} = \frac{\text{Volume of foam after 30 minutes}}{\text{Initial volume of foam}} \times 100 \quad [10]$$

Rennet Coagulation time

The RCT of reconstituted MPC was estimated by rheological method where it is defined as the time in minute when the aggregated system had a storage modulus equal to 1 Pa (Lucey, 2002). MPC was mixed with deionized water to make 3.5% protein on test solution and stirred using magnetic stirrer at 700 rpm for 30 min at 22°C. Three different concentration of calcium chloride (CaCl₂, Sigma-Aldrich, assay ≥ 99%); 0.05, 0.1 and 0.25% (wt/wt of test solution) was added and mixed for 5 min and the pH was adjusted to 6.5 with lactic acid at the end. Nine-microliter of rennet (CHY-MAX® Extra, CHR HANSEN, material no. 73812, activity: ~650 IMCUML⁻¹) was mixed in 60 mL test solution and then run in rheometer (MCR 92, Anton Paar Ltd., UK) with bob-and-cup configuration at constant strain of 0.5% with a frequency 1 Hz at 32°C. A strain (γ) sweep test was conducted until the storage modulus (G') reached to 1 Pa. As a reference, pasteurized skim milk having protein content ~3.5% was run in the same system instead of reconstituted MPC for three times.

Statistical Analysis

All the data from three replications were analyzed statistically using Agricolae statistical package for Agricultural Research in R programming language (version 3.5.2), created by R Core Team. Tukey's HSD test was used for determining the differences between the treatment means, which were confirmed significant when $P < 0.05$.

RESULTS AND DISCUSSION

Milk protein ingredient has both nutritional and functional role in food product development. Functional role of protein ingredients depends on pH, temperature, ionic strength, sugars, and processing temperature during product formulation. The native structure of proteins may change after the interactions between protein molecules and impacts on the functionality (Singh, 2011). We utilized plate and frame filtration (PF) to manufacture high solids milk protein concentrate (MPC) and dried in spray drier to get MPC powder. The composition of MPC powders has shown in Table 1. Moisture content of the MPC powder was in the range of 2.04 to 3.51% which was under the limit ($\leq 6\%$) as described by U.S. Dairy Export Council. Result showed that high temperature PF had impacted significantly ($P < 0.05$) on the moisture content of the powder. High solids level in feed gives lower moisture product after spray drying because of having smaller proportion of water to be evaporated in same environment compared to lower feed solids, hence high chance of getting low moisture product (Sharma et al., 2012).

Physicochemical Properties

Wetting time. The wettability of MPC powders were measured as a rate of complete wetting when placed on the surface of still water at specified temperature. Poor wetting

powders be likely to float on the surface of still water and be submerged very slowly whereas the good wetting powders sink fast into the water. The wetting time of MPC powders measured at 22- and 50°C were in the range of 62 to 136 min and 19 to 50 min, respectively (Table 2). Results showed that PF increased the wetting time of MPC powders significantly ($P < 0.05$). The longest wetting was recorded for the powder from PF50HS treatment. According to Silva and O'Mahony (2017), the wetting properties of milk protein ingredients were affected by CN and ionic calcium contents in the powders. The protein fractions mainly α - and β -CN show hydrophobic nature which prevent the movement of water into the powder particles (Havea, 2006, Crowley et al., 2015). The poor wetting properties of MPC powder is also due to the formation of a crust by fusion of casein micelles on the surface of the powder particles (Fyfe et al., 2011). Surface composition of the milk powder particle also affected in the wettability (Hussain et al., 2011), which is agreed to our results for which the MPC powder samples from high temperature PF treatments had poorer wettability. In the powder from high temperature PF, with increasing protein content, the level of soluble components such as lactose and minerals (total ash) were decreased (Table 1) resulted longer wetting time. Surface composition can also be influenced by the bulk composition and may impact on the wettability of the powders Fitzpatrick et al., (2017). Similarly, the lower density of MPI powder may also contribute to its poor wettability. The wetting time of MPC powders was reduced nearly three times when the test was carried out at 50°C. But Fitzpatrick et al., (2017) observed different results when studied in MPI powder and found that increasing temperature did not improved the wettability of the powders and described that

some powders form a strong film at powder/water interfaces which might act as a barrier to water penetration hence poor wettability.

Heat Coagulation Time. Heat stability of reconstituted MPC was tested as HCT, which was in the range of 19.89 to 23.26 min (Table 2). High temperature PF had significantly ($P < 0.05$) lower HCT. Singh (2004) and Renhe and Corredig (2018), mentioned that HCT was decreased with increasing protein concentration. Bovine milk at its normal pH (~6.8) can usually tolerate heat treatments at 140°C for up to 20 min so that the heat stability of normal milk will not be a problem in dairy processing. But at high protein milk such as MPC, it may not withstand that high temperature, so, HCT is carried out at 120°C (Fox, 1981). Caseins show higher heat stability whereas whey proteins are susceptible to heat. When the amount of whey protein decreased in MPC, the relative amount of casein increased which plays positive role to improve the heat stability. But, when the total amount of protein increased, such as in the MPC powders from high temperature PF, the heat stability could not improve. Next to protein, HCT of MPC powder is also depends on pH. Crowley (2014) found that when the pH increased, the activity of calcium ion decreased which increases the HCT, however the trend was different for the MPC90. In one of the study, the heat stability of MPC retentates from UF were recorded >30 min (Renhe and Corredig, 2018), which showed that the heat stability can be decreased after spray drying when compared with the HCT of feed sample from the current study (Table 2). The HCT can be affected by processing time and temperature, stage of lactation, whey protein to casein ratio and colloidal calcium phosphate concentration (Fox and Morrissey, 1997). It was also reported that the reduction in particle size by physical means such as homogenization, cavitation etc.

reduced the HCT to some extent (Deysher et al., 1929, Dahiya, 2016). As described by Iordache and Jelen (2003), a whey and casein proteins complex can be formed during high temperature processing which is sensitive to secondary heat induced coagulation, which was in agreement to our current HCT results for the high temperature PF treatments.

Relative dissolution index. The dissolution capacity of the MPC powders were analyzed for the changes in fine ($<10\mu\text{m}$) counts with time when exposed in water by using focused beam reflectance measurement and the results has shown in Figure 1. The fine particle counts of all MPC powders were increased with time. After starting the experiment, particle dissolution rate of MPC powders from all the treatments was slow and unsteady for the first 7 min. Later, the counts of particles from feed and PF22 treatments was increased sharply until 17 min and then the rate was decreased but for the powders from high temperature PF treatments the counting rate was increased steadily throughout the testing period. As described by Fang et al., (2010), the greater the slope of the chord length plot, faster the dissolution rate of the powder. In the current study, the growth of fine counts was rapid for the powders from Feed and PF22 whereas the counts for the powders from high temperature PF treatments were relatively low. In 30 min, the fine counts of powders from feed, PF22, PF50MS and PF50HS were 77384, 74366, 58716 and 40367, respectively. From the result, it can be predicted that the availability of fine particles were less in the powders from high temperature PF treatments, and there could be the possibility of having bigger particles ($>10\mu\text{m}$) formed by the protein to protein interactions during high temperature PF processing. In gist, the dissolution rate of

MPC powders from low temperature processing was considerably higher than the powders from high temperature processing.

Crowley et al., (2015) and Hauser and Amamcharla (2016) also observed similar trends of fine particles counting when MPC was exposed in water and analyzed under FBRM. When MPC is processed for long time at high temperatures, caseins and whey proteins can be crosslinked to each other which delays hydration capacity of the MPC powder (Anema et al., 2006), and the chance of crosslinking can be increased if the level of protein in the MPC increased which thereby increased the formation of bigger particles showing more resistance to dissolve in water (Crowley et al., 2015), which supported to our results particularly for the high temperature PF treatments. In another study conducted by Babu and Amamcharla (2018) found that higher storage temperature increased protein-protein aggregations in MPC and showed lesser counts of fine particle during FBRM analysis. The dissolution rate of MPC powder from feed material was assumed to be 100% and compared with the dissolution rate of MPC powders from PF treatments and calculated as relative dissolution index (RDI) (Table 2). High temperature PF treatments had a significant impact ($P < 0.05$) on the RDI. Among the PF treatments, the MPC powders from PF22 and PF50HS treatments had the highest (92.73) and the lowest (56.27) RDI, respectively. The RDI value helps to predict the solubility of MPC powders. Higher RDI can usually be correlated to the better solubility of the powder.

Rheological Properties

Reconstituted MPC (10% wt/wt) prepared by stirring with deionized water for 30 min were conducted rheological tests for the parameters such as apparent viscosity, yield stress, consistency, and flow behavior indices, and the results has expressed in Table 3.

High temperature PF had a significant ($P < 0.05$) impact on the apparent viscosity of the reconstituted MPC. Apparent viscosity of reconstituted MPC from high temperature PF treatments were lower when compared to the reconstituted MPC from feed and low temperature PF. Caseins micelle can hold large volume of water when it gets adequately hydrated and become viscous. But extent of hydration depends on time and temperature of water. Results showed that the hydration rate was decreased with increasing level of protein in the dispersion during reconstitution, resulting lower apparent viscosity. The powders from high temperature PF showed poor wettability (Table 2) also verified that it takes longer hydration time. The lower hydration rate of MPC from high temperature PF treatments might be due to lower level of total ash contents (Table 1). So, the viscosity of reconstituted MPC could be different if the hydration were elongated. Meletharayil et al., (2015) compared the water holding properties of MPC gels having protein content between 50 to 85% and found poorer water holding in the gels made by MPC of higher proteins when compared to the gels from MPC having lower proteins, indirectly agreed to our results, where the water holding can be correlated to the apparent viscosity. We also observed the rheological behavior of reconstituted MPC and the trend line of shear rate versus shear stress was best fitted with the Herschel-Bulkley model. Yield stress of reconstituted MPC from all the PF treatments were in narrow range, 1.39 to 1.53 Pa and were statistically similar ($P > 0.05$) but the yield stress of reconstituted MPC from feed-material was significantly ($P < 0.05$) higher (1.95 Pa) than the rest of the treatments. The consistency index of the reconstituted MPC from high temperature PF was decreased which indicated that the apparent viscosity was decreased with increasing shear rate.

The flow behavior index was higher when compared with feed and low temperature PF treatment. Reconstituted MPC from feed exhibited more consistent gel but the flowing behavior was poorer and the MPC gels from high temperature PF showed better flowing behavior though they were less consistent. The disaggregation of the protein particles due to shearing with the rate higher than the normal can go in Brownian motion (Pradipasena and Rha, 1977). We had exposed the reconstituted MPC at the shear rate 1 to 1000s^{-1} resulting the viscosity was decreased in the starting and steadily increased after a point. This kind of shear thinning followed by shear thickening behaviors might be due to structural changes in the protein matrix of MPC solution when shear rate progresses. This information favored to decide that reconstituted MPC from PF system behave as a non-Newtonian time-independent fluid. According to Pevero et al., (2007), the flow behavior index of a fluid is near to 1 if it passes from a shear thinning to a shear thickening nature which generally depends on particle size, shape and distribution in the fluid, which was agreed to the behavior of reconstituted MPCs used in this study. The coefficient of determination (R^2) value was $\geq 97\%$ for all the treatments to fit with the Herschel-Bulkley model.

Solubility

Solubility is assumed as the most important functional property of MPC. Solubility of fresh MPC powders in deionized water was assessed at 22- and 50°C and the results are given in Figure 2A. PF had a significant impact on the solubility when measured at 22°C and 50°C. The range of solubility of MPC powders was in the range 51.90 to 75.60% when measured at 22°C. According to the McCarthy et al., (2014), the differences in solubility of MPC powders might be due to the differences in particle size, way of

mixing, dissolution time and temperature and they found that with increasing time of agitation, the number of fine particles increased, and at higher temperature, the deforming rate of bigger particles also increased which results better solubility. Schuck et al., (2013) also reported that casein-micelles of high protein MPC powders required several hours to release casein from powder particles in the solution at lower temperatures and moderate agitation condition. At high temperature, solubility of MPC powders was increased by >20% compared to room temperature (22°C) and was in the range 74.78 to 91.95%. During the concentration of MPC, the viscosity can be raised significantly with increasing protein content which reduces the solubility after drying (Patel et al., 2007). The poor solubility of MPC powder from high temperature PF treatments might be due to the formation of hydrophobic casein monolayer on the surface of the powder particles at higher processing temperatures as described by the Fyfe et al., (2011). Past studies showed that the particle size of powders can be increased with increasing protein content in the MPC which can have longer rehydration time (Richard et al., 2013, Rupp et al., 2018). However, the solubility of MPC highly depends on the temperature of solvent and force applied in mixing. Zwieters (1992) revealed that the solubility of MPC could be improved by increasing the hydration temperature which increase the rate of water movement towards the center of the powder particles. Similar other studies (Mistry, 2002, Mimouni et al., 2009) also mentioned that solubility of MPC powder highly depends on reconstitution temperature, which is agreed to our result. Solubility of MPC powders from high temperature PF treatments was reduced significantly ($P < 0.05$). High temperature processing applied in the production of MPC reduces the solubility of powders after drying (Anema et al., 2003, Huppertz et al., 2018). When milk is heated

between 4 to 60°C, there are number of reversible physicochemical changes occurs which influences on the solubility of milk powders (DeWit and Klarenbeek, 1984).

The solubility of MPC powders were decreased by 6 to 12% after 6 months of storage at ambient condition when measured at 22°C and the range was 39.00 to 66.33% (Figure 2B). Solubility of MPC powders from high temperature PF treatments was significantly ($P < 0.05$) low. When MPC is stored at ambient or higher temperatures, the micellar components of MPC such as caseins, calcium and phosphorous released slowly when dissolve in water, which reduces the dissolution rate (Mimouni et al., 2010). At higher temperature (50°C), solubility of MPC powders was increased by 9 to 13% compared to room temperature (22°C) and was in the range 48.33 to 79.00% after 6 months of storage. Results showed that, even at higher temperature (50°C) more than half of the particles could not be dissolved within 30 min. Number of researchers mentioned that the solubility of MPC decreases with increasing storage time and temperature. Several past researchers mentioned that high protein MPC powders exhibited rapid decrease in solubility for first two months when stored at higher temperatures (Anema et al., 2006, Rupp et al., 2018). The two major factor claimed for the loss of solubility during storage are the interactions between CN to CN and CN to whey proteins on the surface of powder particles and the rate of interactions increased with increasing protein contents (Havea, 2006, Fyfe et al., 2011, Uluko et al., 2016), agreed to the results of current study.

Emulsifying Capacity and Emulsion Stability

Emulsifying capacity and the stability of emulsion of MPC powders were tested and the results are expressed in Figure 3. Emulsion capacity of reconstituted MPC (1% wt/wt) from all the treatments were found in the narrow range; 49.33 to 55.89%. High

temperature PF did not have a significant ($P > 0.05$) impact on the emulsifying capacity but impacted significantly ($P < 0.05$) on the stability of emulsion. The concentration of protein on the surface of emulsion droplets determine the emulsifying capacity of the protein. Similarly, the total surface protein concentration increased with increasing protein concentration (Ye, 2011). In the current study, the concentration of protein in the MPC powders from high temperature PF treatments was higher which might be the reason for having higher emulsifying capacity when compared with feed or PF22 samples, however the difference was not a significant ($P > 0.05$). In the current research, accelerated test of emulsion stability was done by heating.

The emulsion capacity was reduced by 5 to 7% after heating at 80°C for 30 minutes, hence the emulsion stability was in the range of 44.70 to 48.83%. The stability of emulsion depends on the droplet size of emulsion hence, smaller the droplet size better the flocculation and thereby increases the stability of the emulsion (Dickinson, 2003, Ye, 2011). Ye (2011), also mentioned that the average size of stabilized emulsions decreased with an increase in the protein concentration up to 5%. The stability of MPC also depends on the calcium contents of MPC. Low calcium concentration attributes to reduce the flocculation induced by the protein particles in the aqueous phase, which is the condition for the poor stability of the emulsion (Dickinson and Golding, 1997, Ye, 2011). In the current research, the highest emulsion capacity and the most stable emulsion was performed by the MPC powder from PF50HS treatment. In the related research, we determined that MPC from PF50HS treatment had higher calcium content (data not shown) in comparison to other treatments, which agreed to the findings of past research. Food emulsion is very complex in its structure and can be changed in many ways such as

creaming, flocculation or their combination (Dickinson and Golding, 1997). Stability of an emulsion rely on the balance of attractive, repulsive, stearic and depletion forces, and for the better stability, the repulsive forces need to be greater than other forces (Dickinson, 1997). The increased level of denatured whey protein in the emulsion also improves the stability of emulsion (Britten and Giroux, 1991). The stability of emulsion for the feed and PF22 treatments was lower, which might be due to the lower protein content as well as lower level of denatured protein content in the MPC from those treatments when compared to the stability of emulsion from high temperature PF treatments.

Whey Protein and Denatured Protein

Whey protein content in the MPC powder was determined by the difference of NCN and NPN (Dupont et al., 2011) and results has shown in Table 4. Whey protein was found in the range 13.65 to 20.53% based on the true protein available in the powder. High temperature PF impacted significantly ($P < 0.05$) on the level of whey protein in MPC powders. The ratio of casein to whey protein in the milk can be varied with season (Heck et al., 2009, Chen et al., 2014). It is important to know the level of whey protein in the MPC because it is interconnected with the functional properties of MPC. The level of whey protein in the milk varies with lactation and season and its existence level in the product depends on the temperature and time of processing. High temperature processing causes denaturation of whey proteins and casein to form protein aggregates, and past studies showed that longer the exposure higher the aggregation (Qian et al., 2017). During MPC production, we heated the milk in different steps such as pasteurization, preheating, high temperature PF processing, and spray drying, which added the level of

protein denaturation in every step and increased the protein aggregation. So, the availability of whey protein was low at high temperature PF treatments. The level of aggregation impacted number of functional properties of protein rich dairy powders (Britten and Giroux, 1991, Hupertz et al., 2018, Oldfield and Singh, 2005). The level of denatured protein was found in the range of 0.14 to 11.28% based on total protein. High temperature PF increased the level of denatured protein significantly ($P < 0.05$). The slight reduction of whey protein or small increment in the denatured protein in the PF22 treatment when compared to the feed might be due to the shear-induced denaturation in multiple recirculation in PF processing, however the level was statistically similar ($P > 0.05$). Rupp et al., (2018) also mentioned similar results when MPC80 was concentrated by evaporation at 55°C. Our results showed that the level of denatured protein was higher in PF50MS and highest in PF50HS treatment when compared to PF22 treatment. Results verified that longer the heating more was the denaturation.

The denatured whey protein nitrogen has poor solubility with water (Patel et al., 2007). According to ADPI (2016), non-fat dry milk (NFDM) powders are classified as high-heat, medium heat and low-heat based on the availability of water soluble whey protein nitrogen and must have >6 , 1.5 to 6, and $<1.5 \text{ mg g}^{-1}$ powder, respectively. Based on this category, all MPC powders used in this study can be compared with the low-heat NFDM. Heating milk proteins for long time could increase the interactions of hydrophobic groups in the interaction of proteins mainly between β -LG and κ -CN (Patel et al., 2006), which results higher amount of denatured proteins, agreed to the results of current study. Ferrer et al. (2008) found that native whey protein was denatured in high level in MPC90 and MPC70 compared to MPC56 when tested for their characterization.

Foaming Capacity and Foam Stability

Milk proteins have strong affinity to adsorb at air-water interface to develop foams and helps in their stability (Dickinson, 2003). The foaming properties of dairy ingredients are important for the products in which air–water dispersions are essential. Foaming capacity of milk proteins can be correlated to their ability to develop air–water interface to create a foam in a desired condition (Singh, 2011). The foaming property of a protein rely on the surface activity (phillips, 1987). In the current study, high temperature PF had a significant ($P < 0.05$) impact on the foaming capacity of MPC powders and was in the range 128.00 to 144.67% (Figure 4). Foaming capacity of low temperature PF (106.77%) and feed (95.34%) were statistically similar ($P > 0.05$). Several studies have shown that the foaming behavior of proteins is dependent on factors such as heat treatment, pH, and ionic environment influence (Ward et al., 1997; Hagolle et al., 2000; Zhang and Goff, 2004). Results of the current study showed that foaming capacity was impacted directly by the level of protein in the powder. However, Huppertz (2010) described that MPC having higher amount of soluble protein shows better in foaming. The ratio of casein to whey protein in the milk can be seasonal (Heck et al., 2009, Chen et al., 2014). Both milk protein content and casein to whey protein ratios are known to change with the season (Chen et al., 2014) which can impact on the foaming capacity.

In protein foaming, air as well as water vapor is forced into the protein solution to build a foam. When a foam is formed, the polar nature of milk protein draws water at one end where it repels in the next end, the phenomenon helps to maintain the stability of foam (Marinova et al., 2009). MPC powders from this study demonstrated very good foaming capacity as well as foam stability. The stability of the foam from high

temperature PF, ranged 91.97 to 93.00%, was similar ($P > 0.05$) to the feed (95.72%) (Figure 4). The foam stability was slightly lower for the PF22 treatment (86.67%). When milk protein level increased between 1.5 to 4% in the protein dispersions, the stability of foam increased (Xiong et al., 2020). During foaming, caseins may go in self-association due to hydrophobic interactions and the association may increase with increasing temperature and ionic strength (Schmidt et al., 1972). Yankov and Panchev (1996) found MPC was poorer in foaming capacity and stability when compared to whey protein concentrate, and similar information has been provided by Singh (2011) in the study of functional properties of milk proteins. deWit and Klarenbeek (1984) mentioned that whey protein denaturation would be irreversible when milk is heated between 60-100°C, which improves the foaming property however the stability of foam depends on the pH. Similar information has been provided by Mistry and Hassan (1991) on their study on delactosed high milk protein powder. In another study, it has been described that the stability of foam strongly improved when the denaturation of β -LG increased (Bals and Kulozik, 2003). Most of the abovementioned past studies described that foaming capacity of milk protein increased with protein content when measured at neutral pH and the stability of foam can be higher with increasing whey protein and or denatured whey protein contents, which is in agreement to our results.

Rennet Coagulation time

MPC powders were tested for the RCT in their reconstituted form with 3.5% level of protein. Coagulation time was noted until the storage modulus (G') become 1 Pa in a rheological test (Lucey, 2002) and compared with the RCT of pasteurized skim milk (protein content ~3.5%) run in the same system and the result has shown in Table 5. The

RCT of reconstituted MPC from different PF treatments was higher compare to skim milk and feed sample. Reconstituted MPC from high temperature PF treatments were remained uncoagulated for at least 2 h in the absence of CaCl_2 . But RCT for the reconstituted MPC processed at low temperature PF was 80 min whereas for the feed sample, it was about 1 h. MPC processed from PF had longer RCT compared to the feed and among the PF treatments, MPCs from high temperature PF had significantly ($P > 0.05$) higher RCT in the absence of external CaCl_2 . Mishra and Metzger (2020) observed the RCT of MPC80 concentrated using NF at 50°C was >2 h when tested without CaCl_2 , which was comparable to the result of our current study. Similarly, they also mentioned that the RCT of MPC80 concentrated using NF at 22°C was 14 min when tested in the presence of 0.1% CaCl_2 which is also comparable to the RCT of PF22 treatment (11 min) at similar condition. Ferrer et al. (2008) recorded that the renneting time of MPC containing protein $>70\%$ was approximately 60 min which was comparable to the RCT of feed (56 min) in the current study.

Reconstituted MPC from PF50HS did not coagulate up to 2 h at 0.1% CaCl_2 concentration but coagulated in 99 minutes at the concentration of 0.25%. The coagulation for the PF50MS treatment was appeared in 104 min at 0.1% CaCl_2 concentration. MPCs from feed and PF22 treatments can be coagulated at 0.05% CaCl_2 within the time equivalent to the skim milk without CaCl_2 . Similarly, MPC from PF50MS treatment showed similar RCT effect as of skim milk after adding 0.25% CaCl_2 and for the PF50HS treatment, the dose of CaCl_2 was needed higher than 0.25%. Results indicated that long exposure of MPC at high temperature increases its renneting time, so, dosing of CaCl_2 is to be increased for the appropriate renneting action and shortening of

coagulation time. According to Ferrer et al. (2008), the level of aggregation between whey and κ -casein increased with increasing heating time and temperature due to the denaturation of whey protein. They also mentioned that the amount of macropeptide decreased with increasing the level of protein in the powder which might elongate the renneting time. Milk protein coagulation with rennet occurs in two stages: hydrolysis of κ -CN, and aggregation of coagulated casein Van Hooydonk et al., 1987; Calvo et al., 1995, and both of those coagulation stages impacted by the heat-induced denatured protein in the MPC from temperature PF. During renneting, the aggregation of casein micelles depends on the level of calcium in colloidal or soluble form (McMahon et al., 1993). Ionic calcium accelerates the protein aggregation rate and improves the activity of proteases and thereby increase renneting efficiency (Wolfschoon-Pombo, 1997, Fox et al., 2004). In the current research, with increasing protein content in the MPC from high temperature PF, there is high chance of increasing colloidal calcium and decreasing soluble calcium which impacted for delaying in the coagulation of casein, hence showed higher RCT. We had added CaCl_2 in the reconstituted MPC to increase ionic calcium concentration. The combined effect of increased protein content and high temperature processing for longer time might be the reason for higher RCT for the MPC from high temperature PF, for which the RCT can be shortened by using optimum level of CaCl_2 .

CONCLUSIONS

MPC concentrated using PF system at different temperatures were studied for their functional properties after spray drying. The wetting time of MPC powders was reduced nearly three times when the test was carried out at 50°C. High temperature PF slightly reduced the HCT of MPC powders. Relative dissolution index of MPC powders from

PF22 and feed were comparable, but it was low for high temperature PF treatments. Reconstituted MPC gels (10% wt/wt) showed shear thinning followed by shear thickening behaviors and the trendline of shear rate versus shear stress was best fitted with the Herschel-Bulkley model. Gels from high temperature PF showed better flowing behavior but the flow was less consistent. MPC powders from high temperature PF were less soluble compare to feed and low temperature PF and the solubility was decreased by 6 to 12% after 6 months of storage at ambient condition. Emulsion capacity of reconstituted MPC was similar for all the treatments but the capacity was reduced by 5 to 7% after heating at 80°C for 30 minutes. Protein denaturation in the powders was increased with increasing processing temperature and time. Foaming capacity and foam stability were higher for the powders from high temperature PF treatments. RCT of reconstituted MPC from PF22 treatment was comparable to the feed. Reconstituted MPC from high temperature PF required $\geq 0.25\%$ CaCl_2 to coagulate as skim milk. Overall, this study concluded that temperature optimization is important for concentrating MPC in PF system to maintain the functionality and MPC from high temperature PF requires longer rehydration to make it more functional.

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Table 1. Mean (n=3) compositional analysis of MPC powders

Treatments ¹	Moisture (% wt/wt)	Total Prot (% db)	Total Ash (% db)	Lactose (% db)	Crude Fat (% db)	NPN ² (% db)	NCN ² (% db)
Feed	3.51 ^a	81.77 ^b	7.71 ^a	7.52 ^a	2.72 ^c	0.09 ^a	2.70 ^a
PF22	3.41 ^a	83.04 ^b	7.12 ^b	7.00 ^a	2.92 ^c	0.07 ^a	2.51 ^{ab}
PF50MS	2.32 ^b	87.37 ^a	4.97 ^c	4.37 ^b	3.16 ^b	0.07 ^a	2.24 ^{bc}
PF50HS	2.04 ^b	88.13 ^a	4.91 ^c	4.08 ^b	3.43 ^a	0.06 ^a	1.93 ^c

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively.

²NPN=non-protein nitrogen; NCN=non-casein nitrogen

Table 2. Physicochemical analysis (mean, n=3) of MPC powder¹

Properties	Feed ²	PF22 ²	PF50MS ²	PF50HS ²
Wetting Time at 22°C (min)	62 ^c	89 ^b	99 ^b	136 ^a
Wetting Time at 50°C (min)	19 ^c	33 ^{bc}	41 ^{ab}	50 ^a
HCT ³ at 120°C (min)	25.58 ^a	23.26 ^b	21.50 ^c	19.89 ^c
Relative Dissolution Index	100 ^a	92.73 ^b	76.02 ^c	56.27 ^d

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹MPC powder was prepared by spray drying of retentates from different PF treatments

²Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk);

PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and

PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively.

³HCT=Heat coagulation time of reconstituted MPC (10% wt/wt) measured in oil bath at 120°C.

Table 3. Rheological properties analysis (mean, n=3) of reconstituted¹ MPC

Treatments ²	Apparent Viscosity ³ (cP)	Parameters for the Herschel-Bulkley model ⁴			
		Yield stress (Pa)	K	N	R ²
Feed	17 ^a	1.95 ^a	0.017 ^a	0.95 ^d	0.99
PF22	14 ^{ab}	1.53 ^b	0.010 ^b	1.06 ^c	0.98
PF50MS	12 ^b	1.43 ^b	0.008 ^c	1.15 ^b	0.98
PF50HS	8 ^b	1.39 ^b	0.007 ^c	1.27 ^a	0.97

^{a-c}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹Reconstituted MPC80 containing 10% total solids (wt/wt).

²Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively.

³Viscosity measured at shear rate 1 to 1000s⁻¹ in bob and cup configuration.

⁴Herschel-Bulkley model; $\bar{\sigma} = \bar{\sigma}_0 + K(\gamma)^n$, where, $\bar{\sigma}_0$ is the yield stress, $\bar{\sigma}$ is the shear stress (Pa), γ is the shear rate (s⁻¹), K is the consistency index (Pa.sⁿ) and n is the flow behavior index.

Table 4. Mean (n=3) whey protein and denatured protein of MPC powders

Treatments ¹	Whey Protein (% of true protein)	Denatured Protein (% of total protein)
Feed	20.53 ^a	0.14 ^c
PF22	18.83 ^a	0.59 ^c
PF50MS	15.85 ^b	8.31 ^b
PF50HS	13.65 ^c	11.28 ^a

^{a-c}Values with the same superscript within a column are not significantly different ($P > 0.05$).

¹Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively

Table 5. Rennet coagulation time (minutes, mean, n=3) of reconstituted¹ MPC at different CaCl₂ concentration

CaCl ₂ (%)	SKM ²	Feed ³	PF22 ³	PF50MS ³	PF50HS ³
0	21	56 ^c	80 ^b	>120 ^a	>120 ^a
0.05	2	19 ^c	28 ^c	>120 ^a	>120 ^a
0.1	<1	8 ^c	11 ^c	104 ^b	>120 ^a
0.25	<1	3 ^c	4 ^c	19 ^b	99 ^a

^{a-c}Values with the same superscript within a row are not significantly different ($P > 0.05$).

¹Reconstituted MPC containing 3.5% protein wt/wt basis.

²Pasteurized skim milk (protein content approximately 3.5% wt/wt).

³Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively.

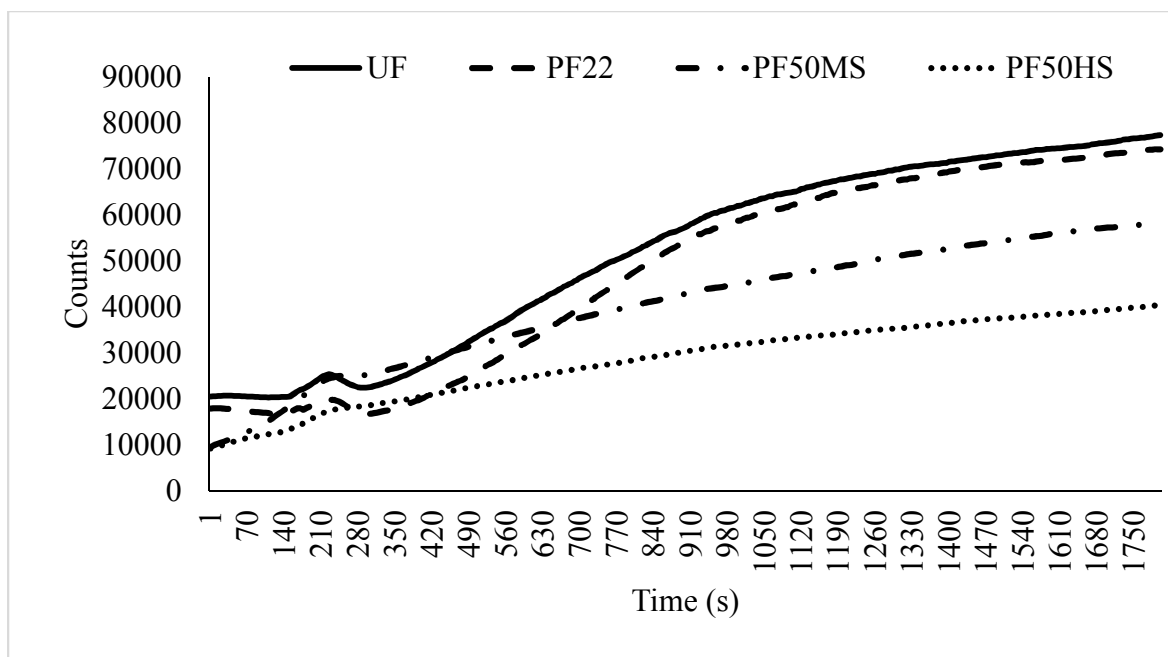


Figure 1. Changes in fine (<10 μ m) counts obtained from the data collected with the focused beam reflectance measurement for MPC powders of different treatments.

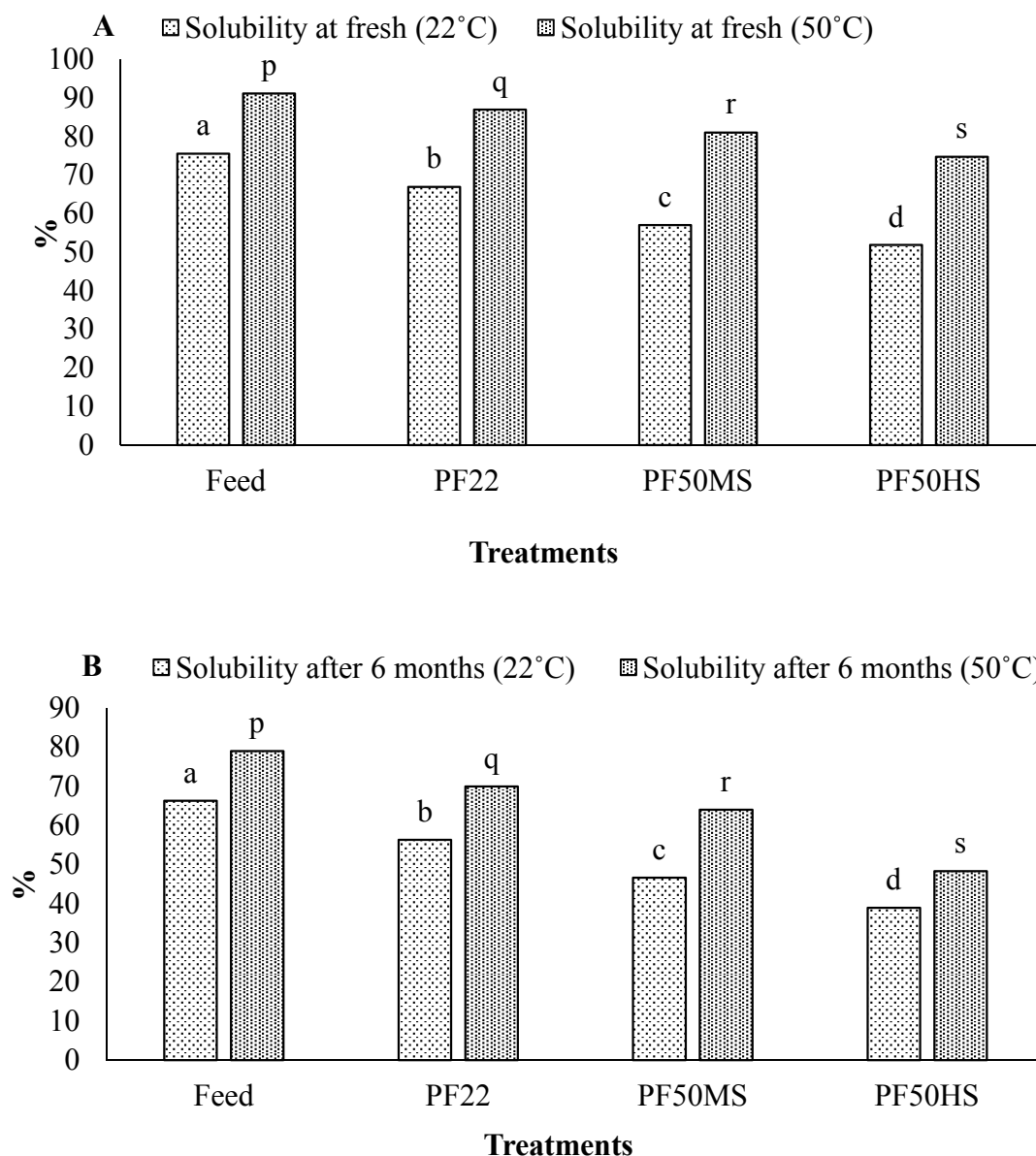


Figure 2. Solubility (%; mean, $n=3$) of MPC powders at fresh (A) and after 6 months of storage at room temperature (B). Solubility was measured in reconstituted MPC solution (5% wt/wt) with distilled water at 22 and 50°C. ²Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively. Values with the same letters (a-d for 22°C and p-s for 50°C bars) are not significantly different ($P > 0.05$) across all treatments.

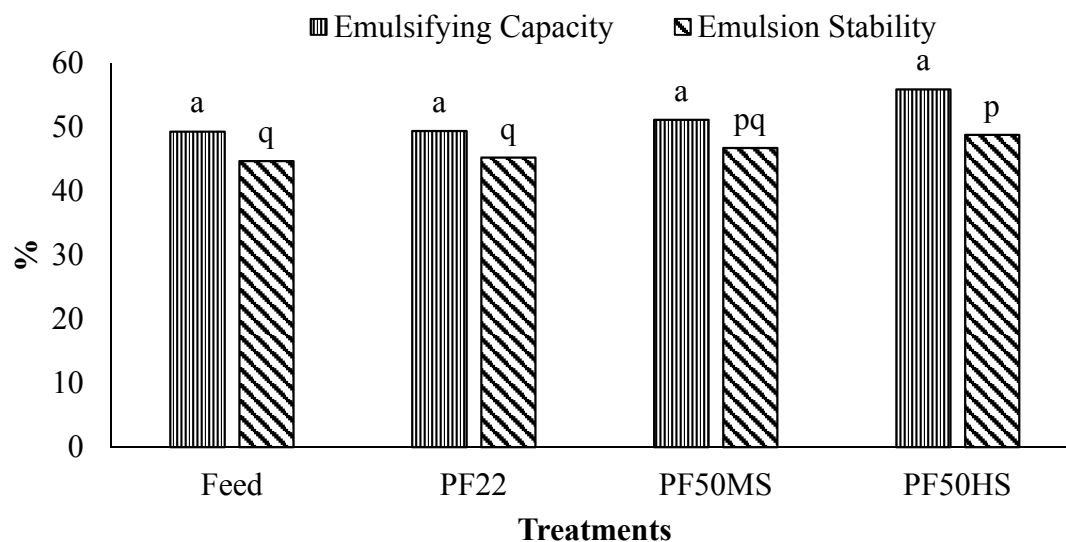


Figure 3. Emulsifying capacity (% , mean, n=3) and emulsion stability (% , mean, n=3) of MPC powders. Emulsifying capacity was measured by mixing sunflower oil (30% wt/wt) with the MPC solution (1% wt/wt). Emulsion stability was estimated after heating the emulsion at 80°C for 30 min. ²Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively. Values with the same letters on the bars of emulsifying capacity or emulsion stability are not significantly different ($P > 0.05$) across all treatments.

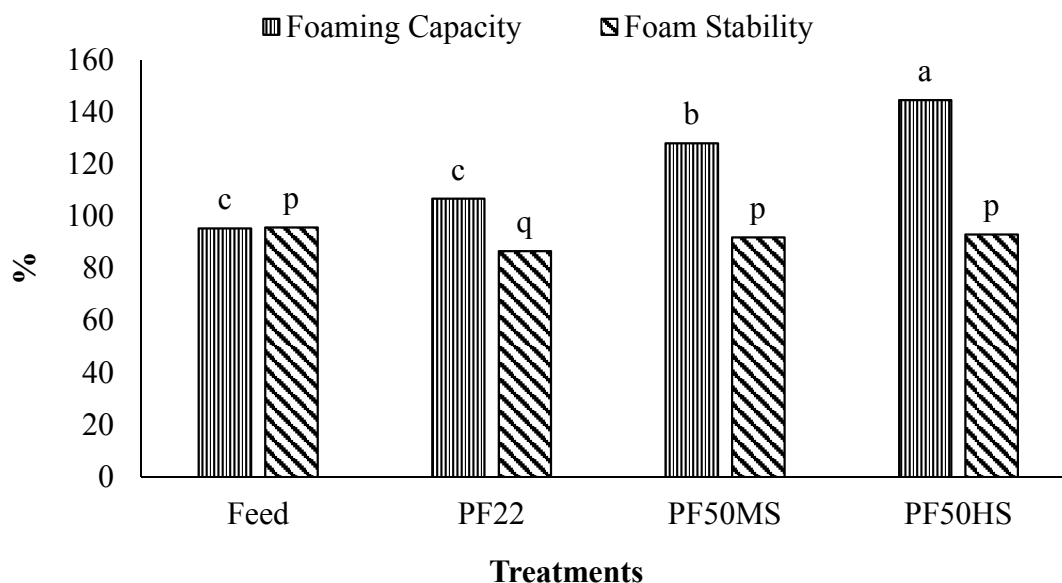


Figure 4. Foaming capacity (% v/v, mean, n=3) and foam stability (% v/v, mean, n=3) of MPC powder. Foaming capacity was measured in reconstituted MPC solution (3% wt/wt) with phosphate buffer (0.05 mol/L, pH=7.0) at 22°C. Foam stability was observed after 30 minutes. ²Feed was an MPC80 powder processed from the ultrafiltration of skim milk (UF-milk); PF22 was an MPC powder from plate and frame filtration of UF-milk at 22°C; and PF50MS and PF50HS were the MPC powders from plate and frame filtration of UF-milk at 50°C for medium and high solids, respectively. Values with the same letters on the bars of foaming capacity or foam stability are not significantly different ($P > 0.05$) across all treatments.

OVERALL CONCLUSIONS

Past studies showed that, concentrating MPC by NF is superior to evaporation in terms of cost as well as functionality of MPC powder after drying. However, issues associated in NF such as concentration polarization and viscosity are always limiting the efficiency of NF. Past studies also described that increased temperature could reduce the viscosity of high solids MPC, which also helps to break the barrier layer formed due to concentration polarization on the surface of NF. Previous research also mentioned that application of HC helps to reduce the viscosity, improves the functionality, and lowers the bacterial load of MPC. So, application of elevated temperature or HC or both can have great potential to increase the efficiency of NF during MPC80 concentration by reducing viscosity of feed and thereby concentration polarization during NF. Similarly, application of UF in PF module can handle the viscous fluids because of the higher transmembrane pressure and crossflow feeding, which can be utilized to concentrate MPC80. The elevation of temperature in PF system may improve the flow of viscous fluids in more efficient way which can be applied to get higher solids MPC80 in the retentate from PF. In this background we had planned to study the impact of increased temperature or HC or both to concentrate MPC80 by using NF or PF system and evaluate the quality of MPC powders after spray drying.

We have studied the implication of HC or high temperature (50°C) or both on the filtration performance of MPC80 in NF system and assessed their effect on the quality. For the control, NF was carried out without HC at room temperature (22°C). High temperature impacted greatly to increase the permeate flux as well as the level of TS whereas HC contributed on increasing the TS in the retentate. Though the level of

microbial load in the retentate was increased at high temperature NF within 4 h of total working, both SPC and mesophilic spores counts in MPC80 powders were within the acceptable limit as described by US dairy standards. Combination of HC and high temperature contributed to increase the tapped density of MPC80 powder which can be associated in the cost reduction during packaging, handling, and storage of the powder. HC also improved the dissolution and foaming characteristics of MPC80 powder. RCT test showed that MPC80 powders from our studies are capable to form cheese curd by adding CaCl_2 in between 0.05 to 0.1%. All MPC powders showed a shear-thinning followed by shear thickening behavior and the trend of shear rate versus shear stress was best fitted with the Herschel-Bulkley model when tested in a 10% (wt/wt) MPC solution. High-temperature NF reduced the solubility of MPC80 powder. Both HC and high temperatures did not impact the flowability, emulsifying capacity, emulsion stability, and WP retention but improved the wettability of MPC80 powder. Heat stability and the level of denatured protein were affected slightly by the combined action of HC and high-temperature NF.

We also studied the impact of high temperature (50°C) in MPC80 concentration using a PF system and for the control, PF was carried out at room temperature (22°C). The increased temperature PF impacted significantly to increase the permeate flux, volumetric concentration ratio, level of TS and total protein to total solids ratio. Spray dried MPC powders were tested for the functionality. Wettability of MPC powders was reduced nearly three times when tested at 50°C. High temperature PF slightly reduced the HCT of MPC powders but did not impact on the emulsion capacity. The dissolution capacity of MPC powder was low for high temperature PF treatments and the insolubility was

increased by 6-12% after storage for 6 months at ambient condition. Foaming capacity and foam stability were improved by high temperature PF treatments. For the appropriate renneting action, reconstituted MPC from high temperature PF required at least 0.25% CaCl_2 . This study determined that high temperatures or their combined action with HC improved NF performance and both HC and NF temperature have important impacts on the functionality of MPC80 powders. We also found that PF can be the potential alternative to concentrate MPC with increasing the level of protein at a time. MPC concentration from either NF or PF at high temperature conditions seemed cost effective because of getting higher solids feed for spray drying. There was at least 4% more TS in the retentate of high temperature PF when compared to high temperature NF which shows PF is more cost effective than NF. However, the level of denatured protein in the retentate of high temperature PF was ~3% more when compared to high temperature NF. So, producers need to select appropriate MPC concentration practices, either NF or PF to meet the users' requirements to fit with their intended products. Overall, this study concluded that temperature optimization is important for concentrating MPC in both NF and PF system.